**CARDIOVASCULAR DISEASE IN AMERICAN INDIANS
(PHASE II)**

OPERATIONS MANUAL - VOLUME ONE

GENERAL DESCRIPTION AND SURVEILLANCE PROCEDURES

THE NATIONAL HEART, LUNG AND BLOOD INSTITUTE
OF THE NATIONAL INSTITUTES OF HEALTH

THE STRONG HEART STUDY

Cardiovascular Disease in American Indians
(Phase II)

Operational Manual

Volume One

GENERAL DESCRIPTION AND SURVEILLANCE PROCEDURES

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MANUAL ONE

GENERAL DESCRIPTION AND SURVEILLANCE PROCEDURES

Table of Contents

APPENDIX

1. General Description and Study Management

1.1 BACKGROUND

1.1.1 General

A review of existing data by the Subcommittee on Cardiovascular and Cerebrovascular Disease of the Secretary of Health and Human Service's Task Force on Black and Minority Health concluded that information on CVD in American Indians is inadequate and strongly recommended epidemiologic studies of this problem (1). The Strong Heart Study is designed to respond to this recommendation.

1.1.2 Scientific Background

A.   Rationale for studying heart disease in American Indians

Although age-adjusted mortality rates for cardiovascular disease are lower in American Indians than in the U.S. population as a whole, cardiovascular disease has become the leading cause of death in American Indians(1). Cardiovascular morbidity and mortality rates may be increasing in some tribes, and the rates appear to differ greatly among various tribes. Cerebrovascular disease is the fourth leading cause of death for American Indians. Age-adjusted mortality rates for cerebrovascular disease were similar to U.S. rates for Oklahoma and Pima Indians and higher for Aberdeen Area Indians in 1981-83 (2).

Several problems have made it difficult to determine the prevalence and severity of cardiovascular disease among American Indians. Small community size, relatively young age, cultural and anthropologic diversity, and the geographic dispersion of the American Indian population have made it difficult to include large numbers of Indians in research examinations and surveys of vital statistics. The relatively low rates of cardiovascular disease in American Indians as a group obscure both regional differences in heart disease (2) and the high mortality rates from heart disease in younger Indians (those aged 25-44 years) (3). The high rates of CVD in younger Indians suggest that the overall CVD rates will increase as the population ages and that CVD may be a more serious health problem among Indians in the future. Definitions of the term "Indian" are variable in published reports, and the denominators from which disease rates were calculated often were based on uncertain estimates of the population at risk. Definitions of disease and methods of its ascertainment have also varied among studies. In addition, health care services available to Indians differ considerably in different geographic areas and possibly contribute to differences in reported rates of cardiovascular disease morbidity and mortality.

The Strong Heart Study was initiated in response to a recommendation by the Subcommittee on Cardiovascular and Cerebrovascular Disease of the Secretary of Health and Human Services Task Force on Black and Minority Health that concluded that information on CVD in American Indians was inadequate.

B.   Description of Strong Heart Study - Phase I

The Strong Heart Study (SHS) is a study of cardiovascular disease among American Indian men and women supported by the National Heart, Lung, and Blood Institute initially for three years from October 1, 1989 to September 30, 1991 (1). The SHS is the largest study of CVD in American Indians ever undertaken. The SHS, which uses standardized methodology, is designed to estimate cardiovascular disease mortality and morbidity rates and the prevalence of known and suspected cardiovascular disease risk factors in American Indians. The study population consists of 13 tribes in three geographic areas: an area near Phoenix, Arizona, the southwestern area of Oklahoma, and the Aberdeen Area of North and South Dakota.

The SHS Phase I included three components. The first was a mortality survey to estimate cardiovascular disease mortality rates for 1984-1988 among tribal members aged 35-74 years. The second was a morbidity survey to estimate incidence of hospitalized myocardial infarction and stroke among tribal members aged 45-74 years in 1984-88. The third was a clinical examination of approximately 4,500 tribal members aged 45-74 years in order to estimate the prevalence of cardiovascular disease and its associations with risk factors. Medical history, family history, diet, alcohol and tobacco consumption, physical activity, degree of acculturation, and socioeconomic status were assessed in personal interviews. The physical examination included measurements of body fat, body circumferences, and blood pressure, an examination of the heart and lungs, an evaluation of peripheral vascular disease, and a 12-lead resting electrocardiogram. Laboratory measurements include fasting and postload glucose, insulin, fasting lipids, apoproteins, fibrinogen, and glycated hemoglobin. Also measured were serum and urinary creatinine and urinary albumin. DNA from lymphocytes was isolated, frozen, and stored for future genetic studies.

The SHS has shown that the three groups of American Indians included in the study are not homogenous with respect to cardiovascular disease and its risk factors. Initial data analysis indicate that the prevalence of ECG diagnosed myocardial infarction varies: among non-diabetic participants, southwestern Oklahoma Indians have the highest (5.8%), followed by Sioux Indians in North and South Dakota (4.5%), and the Pima Indians in Arizona have the lowest (2.9%). For diabetic patients, Sioux Indians have highest rate (10,4%), Oklahoma Indians are slightly lower (9.2%), and the Pimas Indians have the lowest rate (6.3%).

Preliminary analyses of our data indicate that the prevalence of cardiovascular disease (CVD) risk factors also differs from center to center. Diabetes is high in all groups, but highest among the Pimas in Arizona (over 65% prevalence). Mean levels of cholesterol in Sioux and Oklahoma Indians are comparable to those for the U.S. (all races) but considerably lower among the Pimas. The prevalence of smoking is high in the Sioux (approximately 50%), low in the Pimas and intermediate in Oklahoma Indians. Hypertension is less prevalent than in the U.S. in all groups, but the prevalence is higher among the Pima and Oklahoma Indians than among the Sioux. A high prevalence of sedentary lifestyle exists in all three groups. Prevalence of obesity is high in all three groups and highest in the Pimas. Genetic admixture was determined by interview: over 90% of Pimas are full blood Indian, less than half of the Sioux are full blood, and seventy-three percent of Oklahoma Indians are full blood.

C.   Rationale for Phase II of the Strong Heart Study.

The data confirming marked differences in CVD rates and prevalence of potential risk factors indicate that the SHS provides a unique opportunity to learn more about the importance of specific risk factors in American Indians (2). However, in a cross-sectional study, inferences with respect to etiology are limited. The contribution of various risk factors to the occurrence of CVD in the three groups of Indians will be better understood through a longitudinal study and for that reason, phase II proposes to follow prospectively the cohort of persons aged 45-74 who participated in the Phase I examination.

Comparative analysis of risk factors for CVD morbidity and mortality among the three centers will be done to determine why the rates of CVD differ. Since the Pimas have the highest rate of diabetes (over 65%) but the lowest rate of CVD, the study may identify protective factors that will have important implications for prevention of CVD (4). The SHS provides a unique opportunity to determine CVD risk factors in diabetic patients, because of the high prevalence of diabetes in all three groups. The high rates of CVD among the Sioux may be related to a high prevalence of smoking, hypercholesterolemia and diabetes in a population that has greater genetic admixture with non-Indians. A longitudinal study will allow these relationships to be defined more precisely in populations with high prevalence rates of obesity and diabetes and variable rates of other risk factors. Phase II of the SHS allows us to monitor changes in risk factor prevalence over time and to calculate incidence rates of CVD, diabetes, and hypertension among a large cohort of American Indian men and women whose CVD risk factors were uniformly assessed in Phase I examination.

Phase II of the SHS has four components:

1.   A continuous mortality surveillance of the target populations (1989 - 1994);

2.   A continuous morbidity surveillance of the Phase I examination cohort;

3.   A re-examination of the Phase I cohort with an abbreviated personal interview, including a 24-hour dietary recall survey, echocardiography, pulmonary function tests, ultrasonography of the gallbladder, tuberculosis tests, peripheral neuropathy tests, additional laboratory tests, and a repeat of most of the tests done in the phase I examination;

4.   Analysis and presentation of results from Phase I and II.

1.2   RESEARCH OBJECTIVES

The objective of the Strong Heart Study Phase II is to continue to obtain estimates of CVD mortality and morbidity rates using standardized methodology as well as to allow comparison of CVD risk factor levels among American Indian groups living in three different areas: Phoenix, Arizona, southwestern Oklahoma, and Aberdeen area, South and North Dakota.

The specific aims of the study are:

1.   To determine cardiovascular disease (CVD) mortality and morbidity rates among American Indian men and women living in three different areas (Phoenix, Arizona; Southwestern Oklahoma; and Aberdeen Area, North and South Dakota) using a standardized methodology.

2.   To determine CVD risk factors for these Indian groups in a longitudinal study that includes a follow-up examination of a cohort of American Indians aged 45-74 at the Phase I examination (1989-1991).

3.   To compare CVD risk factors in the three centers and relate them to differences in the rates of CVD.

4.   To compare risk factors for CVD among diabetic and non-diabetic participants.

5.   To investigate structural and functional cardiac disease in three groups of American Indians by utilizing echocardiography.

6.   To study pulmonary function among this cohort of Indians and to identify risk factors for pulmonary diseases and their relationships to CVD.

7.   To study the prevalence of gallbladder disease (primarily cholelithiasis) and identify its risk factors.

1.3   STUDY DESIGN

Phase II of the Strong Heart Study has four parts; an extension of the mortality surveillance of the study target population from 1989-1994, a continuous morbidity surveillance of the Phase I examination cohort, a re-examination of the Phase I cohort, and analysis and presentation of results from Phases I and II. (See Figure 2).

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[_____[_____[_____[___[____[____[_____[_____[_____]
11/91     7/92         3/93         4/93  7/93  8/93      7/94         7/95
7/96
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_____ Mortality and
                                                          morbidity surveillance
                    _____ Data analysis of Phase I and manual script
                                                          preparation.
            _____ Development of protocol, manual, and data forms.
                                                          Pretesting of forms.

                                    _____ Training session for Phase II examination.

                                                        _____Phase II exam.

                                                                  _____
                                                                  Data Analysis.
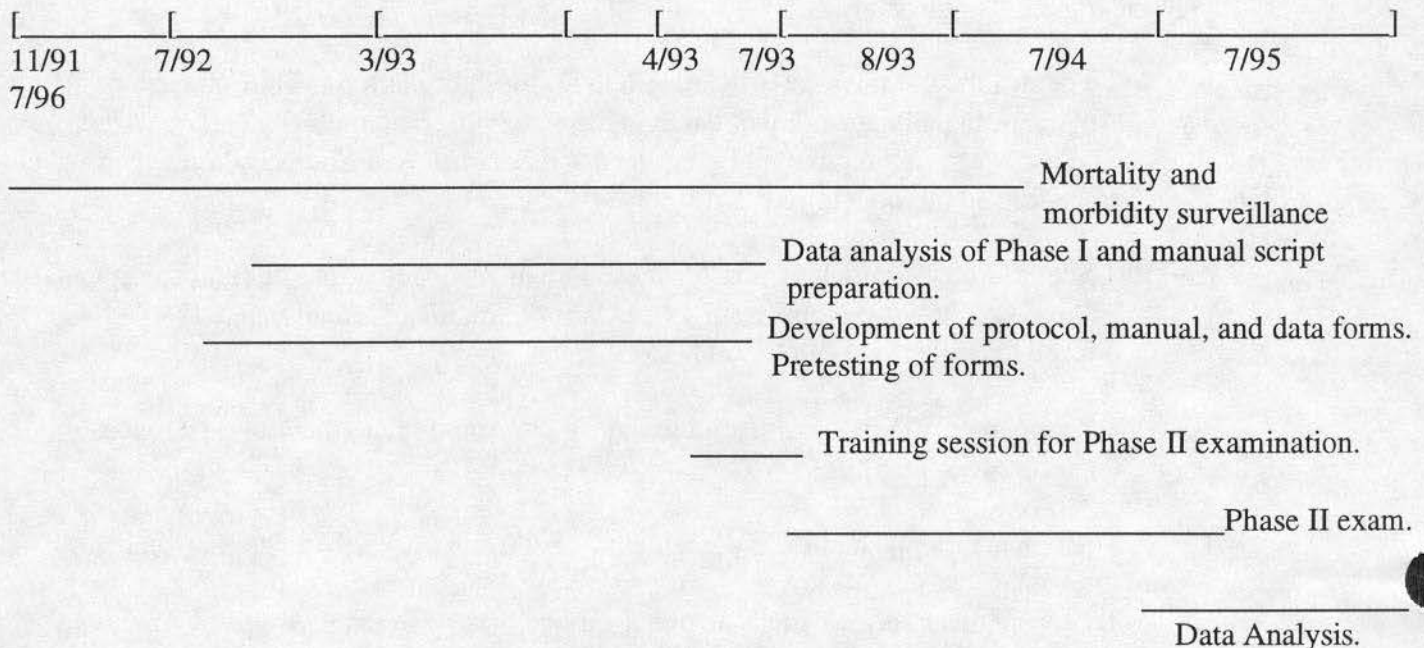
Figure 1   Phase II Timetable

1.3.1 The Mortality Survey

In Phase II of the Strong Heart Study, surveillance activities include annual ascertainment of deaths in the entire target population (which includes the examination cohort) and identification of non-fatal events in the examination cohort.

For the mortality surveillance in the community, procedures similar to those used in Phase I will be continued. All deaths occurring among tribal members aged 35-74 in the three study areas between 1/1/89 and 12/31/94 are first identified through tribal records and other sources. Death certificates will be obtained and coded by a single nosologist. All death certificates with any mention of CVD will be further investigated. Medical records are reviewed and the cause of death confirmed independently. The causes of death of particular interest to the SHS are: myocardial infarction, stroke, sudden coronary death, and congestive heart failure (All participants in the Phase I exam will also be monitored for mortality, regardless of age).

1.3.2 The Morbidity Survey

Only those persons who participated in the Phase I physical examination will be followed for incident events of cardiovascular disease in Phase II. Because the interval between the Phase I and Phase II examination is relatively short (maximum, 6 years; minimum 1.5 years; mean 4 years), the major point at which the occurrence of new events will be ascertained will be at the Phase II examination. Determining the incidence of events at this time will allow for more thorough collection of data and for obtaining signed consent for review of medical records pertaining to the events of interest. In Phase II, the following incident events will be identified: myocardial infarction, stroke, congestive heart failure, angina, and peripheral vascular disease. The occurrence of coronary bypass surgery, angioplasty, or similar procedures will also be determined. Prevalence of the following conditions/indicators will also be ascertained at the Phase II examination: valvular heart disease, positive cardiac catheterization, positive treadmill test, left ventricular hypertrophy, other left ventricular dysfunction, cardiac wall motion abnormalities, and obstructive lung disease. The same definition and criteria for these events used in Phase I will be used in the Phase II study.

1.3.3 The Clinical Examination

The third component of the study consists of a personal interview, a limited physical examination, and laboratory tests for evidence of prevalent CVD, peripheral vascular disease (PVD) and risk factor assessment. Eligible persons will be participants of Strong Heart Study Phase I. Those eligible to participate will be contacted by the staff at each study center.

1.   Personal Interview

Information on the following factors will be obtained from the personal interview:

(a)   Demographic data
(b)   Tobacco use and alcohol consumption
(c)   Traditional values/culture
(d)   Socioeconomic/stress evaluation
(e)   Medical history, particularly CVD history
(f)   Diet

2.   Physical examination

        The physical examination will include the following procedures:

(a)   Height and weight
(b)   Girth measurements: supine waist (abdominal) girth, erect hip girth, and upper arm
        circumference
(c)   Measurements: of body fat using impedance meter
(d)   Sitting arm blood pressure
(e)   Ankle and arm blood pressures in supine position using the doppler
(f)   A resting 12-lead ECG
(g)   Examination of lungs
(h)   Palpation of posterior tibial and pedal pulses
(i)   Auscultation of femoral and carotid bruits
(j)   Echocardiogram
(k)   Pulmonary function test
(l)   Ultrasonography of the gallbladder
(m)   Assessment of peripheral neuropathy using monofilaments
(n)   Skin testing for TB and coccidioidomycosis (AZ center only)

3.   Laboratory measurements:

(a)   Lipids: TC, TG, HDL-C, LDL-C, VLDL-C and VLDL-TG
(b)   Fasting insulin
(c)   Plasma creatinine
(d)   Fasting glucose and 2-hour glucose tolerance test (GTT)
(e)   Urinary albumin and creatinine
(f)   Fibrinogen, plasminogen activator, inhibitor and C-protein
(g)   Glycated hemoglobin ($HbA1_c$)
(h)   DNA extraction and storage
(i)   Red blood cell typing for assessment of genetic admixture

1.3.4   Additional Measurements or Stored Samples from Phase I

        Example:
1.   Plasma Lp(a)
2.   G(m) allotype
3.   LDL size and density subclass

1.4   STUDY QUESTIONS

1.4.1 Mortality Survey

1.   What are the CVD mortality rates (average annual rates for 1988-1994) in the three centers, and how do they compare to one another?

    a)   Mortality rates will be estimated for acute myocardial infarction, stroke, congestive heart failure, total cardiovascular diseases, total mortality, diabetes*, cancer*, and external and other causes*.

    b)   Estimated mortality rates will include the following:

        (1)   Age-specific
        (2)   Sex-specific
        (3)   Age and sex-specific
        (4)   Age-sex adjusted to U.S. population aged 35-74

    *   These will be collected by death certificate only. No attempt will be made to confirm cause of death.

2.   How do these rates compare with reported rates for the U.S. population?

3.   How do these rates compare with reported rates for Indians in these areas?

4.   How do these rates compare among the tribes and among the three centers?

1.4.2 Morbidity Studies

Many of the study questions for Phase II are similar to those asked in Phase I. Some additional research questions of interest, based on Phase II data, include, but are not limited to, the following:

1)   What are the four-year incidence rates of myocardial infarction, cerebrovascular disease (stroke), hypertension, congestive heart failure, large vessel peripheral arterial disease (PAD), diabetes, IGT, and hyperlipidemia?

2)   Which risk factors have the strongest association with CVD incidence and prevalence?

3)   Are the associations between risk factors and CVD similar in all three centers?

4)   Are risk factors for CVD or PAD different among diabetics and non-diabetics?

5)   Have the distributions of any risk factors changed over the follow-up period? Are the changes related to the development of CVD, PAD, diabetes, or IGT?

6)   What is the rate of progression from IGT to diabetes mellitus?

7)   Are diabetes and hyperinsulinemia independent risk factors for CVD?

8)   Are diabetes and alcohol-consumption related to ventricular function as measured by echocardiography?

9)   What is the relationship between ventricular function/mass and other evidence of CVD in this population?

10)   What is the prevalence of rheumatic heart disease as determined by echocardiography?

11)   What is the prevalence of abnormal pulmonary function and how does the prevalence relate to CVD, PVD, diabetes, and IGT?

12)   What are the risk factors for abnormal pulmonary function?

13)   What is the prevalence of gallstones?

14)   What are the risk factors for gallstones?

15)   What are the prevalence of TB and positive PPDs and their risk factors ?

16)   What are the risk factors for peripheral neuropathy ?

17)   What are the differences in diet among the 3 centers as measured by 24-hour recall?

1.5   STUDY MANAGEMENT

1.5.1   Introduction

The Strong Heart Study Phase II is funded by the National Heart, Lung, and Blood Institute, and directed by the Clinical and Genetic Epidemiology Branch, Epidemiology and Biometry Program, Division of Epidemiology and Clinical Applications. The Principal and Co-investigators are listed in Appendix 1 (a). The operations of the study are directed by the Strong Heart Study Phase II Steering Committee, which includes members from each center and the NHLBI Project Manager (see Appendix 1 (b) for the members of Steering Committee). An organizational chart of the Strong Heart Study Phase II is given in Appendix 2. In addition to being a field center, the Oklahoma Center assumes the responsibility of the Coordinating Center, the Dakotas Center is the ECG Reading Center and the Arizona Center acts as the Core Laboratory. Echocardiogram are read at the Cornell University Medical Center under the direction of Dr. Richard Devereux, pulmonary function testing results are analyzed at the Arizona State University under the direction of Dr. Paul Enright. Analysis of the results of gallbladder ultrasonography is directed by Dr. James Everhart of NIDDK. Other key personnel at each center and consultants of the Study are listed in Appendix 3.

1.5.2   Confidentiality of Data

All personnel with access to data collected for the study at each center are required to sign a confidentiality pledge which states that they understand the sensitive and confidential nature of the data and that divulgence of any information will result in disciplinary action. The pledge will be co-signed by the principal investigator. A sample of the confidential pledge is given in Appendix 6 (b).

Completed data forms will be placed in locked file cabinets in offices assigned to the study at each study center and at the Coordinating Center. Only authorized staff members have the key to the office and access to the data forms.

Data on computers at the Coordinating Center will be safeguarded by a password which is known only to authorized personnel.

1.5.3   Communications

1.   Newsletter:

The Coordinating Center prepares and distributes a quarterly newsletter to facilitate communication among Study staff. In general, each edition includes: (1) reports from the Program Office, the Coordinating Center, the Core Laboratory, the ECG Reading Center, Echocardiography Reading Center, Pulmonary Function Testing Center, Ultrasonography Reading Center (NIDDK), and the Steering Committee, (2) a description of the facilities and staff of one field center or central agency, (3) general information on data management and (4) a calendar of events. The newsletter also provides reports on issues such as recruitment and participant follow-up rates, the development and use of new equipment, and preliminary study results and abstracts.

2.   Electronic Mail:

FAX will be the major electronic mail facility to be used by all field centers, the Coordinating Center, Core Laboratory, ECG Reading Center, Echo Reading Center, Pulmonary Function Testing Center, Ultrasonography Center and the Program Office. This electronic mail network will allow rapid and efficient communication among centers for messages such as announcements, meeting agendas, abstracts for clearance and acknowledgements of receipt of data.

3.   Field Center Visits:

The Program Office and Staff from the Coordinating Center, ECG Reading Center, Echo Reading Center, Pulmonary Function Testing Center, Ultrasonography Center and Core Laboratory conduct periodic monitoring visits to field centers as needed to: (1) maintain channels of communication with field center investigators and staff, (2) monitor participant recruitment and surveillance procedures, (3) monitor adherence to the protocol, and (4) provide technical support for activities such as data management and quality control.

1.6 DATA MANAGEMENT AND STATISTICAL ANALYSIS

1.6.1 Data Forms and Guidelines for Completing Forms

Uniform data entry forms for all information to be collected will be designed by the Coordinating Center for use by each Study Center. Each study subject will have a unique identification number (ID number). Please see the Strong Heart Study Phase I Manual page 12a for the detailed procedure to assign the study ID number. For those who return for the second phase examination, the original ID number assigned in the Strong Heart Study Phase One will still be used. The ID number will be stamped on every page of all forms at each center. For laboratory specimens, printed labels supplied by Computype Inc. will be used.

Standard IHS community codes will be used to identify the community where the participant resides. A list of community codes for the three centers is given in Appendix 4. Hospitals where the subject died or were treated for CVD are also coded. Standard IHS facility codes will be used to identify IHS hospitals and clinics. Codes for other non-IHS hospitals will be assigned by each center. The hospital/clinic codes are given in Appendix 5. In addition, every member of the Study is assigned a Personnel Code which will be used to identify the person who filled out a specific data form. The Personnel Codes for the three centers are listed in Appendix 6 (a). Additional Codes will be added sequentially as new employees begin to work on the project.

All data forms must be filled out legibly and completely. Each and every form will be reviewed and checked for completeness and legibility before it is sent to the Coordinating Center. The following are a few guidelines for form completion:

1. All forms should be filled out in black pen. Print all information in block capital letters, with one letter only in each box, so that keypunch errors can be minimized. For example, one should differentiate: 7 from 1, U from V, 4 from 6, P from D, M from N, C from O, and T from J.

2. For names and addresses, start from the leftmost box and leave the unused boxes blank. Include periods for initials.

Example:

Name: | S | M | I | T | H | | J | R | . | | J | O | H | N |

If the address is a post office box or rural route, record in the field for "street number", as

| R | T | | 5 | , | | B | O | X | | 5 | 4 | A | |

or

| I | 4 | 7 | 3 | | S | T | A | R | | S | T | R | E | E | T |

3. For numerical values, fill in the boxes in a right justified manner and leave the unused boxes blank.

Example:

Triglyceride:

| | | | | I | 6 | 7 |

4. For dates, two digits are allowed for each of the month, day and year. If the number has only one digit, use zero in front of the number.

Example:

Date of birth:

| 0 | 8 | / | 0 | 7 | / | 3 | 8 |
| mo | | day | | | yr |

5. When recording dates, use 99 for missing months, days or years.

6. To correct an error, draw a single line through the mistake and write the correct value above.

Example:

Age at Death:

| ~~6~~ 7 | ~~4~~ 4 |

7. Fractions should be rounded up to the nearest whole number if the fraction is 0.5 or more, otherwise, drop the fraction, e.g. $2.25 = 2$; $2.75 = 3$; $3.5 = 4$.

8. If an interval is given, record the midpoint of the interval if it is a whole number. If the midpoint includes the fraction 0.5, use the rounding rules previously given.
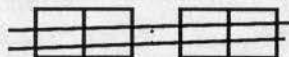
Example:

16-18 months, midpoint = 17 months, record 17.
13-14 months, midpoint = 13.5 months, record 14.

9.   Unless otherwise instructed, no item on any of the forms should ever be left blank. Codes to be used in the event of missing or incomplete data are given under the heading of each specific item. If there is not a code for the "unknown" category, draw two parallel lines horizontally through the box or boxes to indicate that the interviewer or abstractor did not ignore the question. For example, if the time of death is unknown, do the following:

Time of death:

1.6.2   Monthly Mailings Of Data To The Coordinating Center

The Arizona Study Center will cease data collection at the close of operations on the first Friday of each month and prepare all recently collected data for shipment to the Coordinating Center. The Oklahoma Study Center will do the same on the second Friday of the month, as will the South Dakota Study Center on the third Friday of the month. This will allow for modular migration of data files to the Coordinating Center.

Preparation of the data for shipping will require a review of each data form for completeness (i.e. no missing responses or miscoded entries). Legible photocopies will be made of all original data forms. The photocopied forms will be separated by form type and sorted in ascending order by ID number. These forms should be packaged in a mailer made of corrugated cardboard and secured with the type of mailing tape that has fibre threads running through it. Each mailer should be labeled with large legible printing of the following address:

> Strong Heart Study Coordinating Center
> Center for Epidemiologic Research
> University of Oklahoma Health Sciences Center
> 801 NE 13th Street
> College of Health Building, #317
> Oklahoma City, OK 73104

This label should be covered with clear adhesive tape to protect the label from moisture. The original data forms associated with this mailing should not be filed until the Coordinating Center acknowledges receipt of the photocopies. In the event that forms are lost in the mail, the Study Center must photocopy the originals a second time and repeat the above processing.

1.6.3 Procedures for data entry and verification of completeness

Each field center examines every data form for completeness and accuracy before sending it to the Coordinating Center. The Coordinating Center logs each form, laboratory result, and ECG report received in the Participant Forms Logbook. This is manually done. The ID number, participant's name, and date that the item is received are recorded. At the same time, the completeness of each form is checked. All the incomplete items (missing, questionable, unclear) are recorded and the corresponding field center is contacted to find out the reason. If the missing information can be obtained with additional effort, the form is returned to the field center. It may take several months for the field center to collect the missing information since it is very difficult to recall the participants. When these items are completed by the individual center and received by the Coordinating Center, the logbook is updated. Before the ECGs are Minnesota Coded, for interim reports and quick reference, both the machine reading and cardiologist's reading are coded according to the CAPOC MUSE Library Statement. Photocopies of the nosologist's codings of cause of death are made and sent back to each field center.

The complete data forms, ECG reports, and laboratory results are then given to the data manager for entry into the computer. The two data entry persons enter the data separately and exchange what they have entered and reenter. The two sets of the data are compared to identify data entry errors. Any inconsistent items are checked against the original data form to find out which one is correct and who made the mistake. After all the corrections are made, the error-free file is then appended to the permanent file which is used for data analysis. The lipid and glucose data received from the Core Laboratory on diskettes as ASCII files are directly converted into SAS datasets. However, before these data are merged into the permanent data files, they are checked against the values given by the laboratory on paper to ensure the conversion is correctly done. After data entry is completed, all the forms are stored in locked file cabinets.

After all the available data from the physical examination are entered into computer, the Coordinating Center also generates a Summary Report for each participant and sends the report to the field center. These summaries are then forwarded to the participant and his/her physician.

1.6.4 Data Backup:

Several backup procedures are used to ensure the safety of the SHS data files.

a.   Daily backup: Two sets of diskettes are rotated to backup the data every day from Monday through Thursday (one for Monday and Wednesday and the other for Tuesday and Thursday).

b.   Weekly Backup: Similar to daily backup, two sets of diskettes are rotated, each for every other week. Backup of the week's data set is done every Friday.

c.   Tape backup: Additional permanent files are stored in the 486-computer, and backed-up every day by rotating two sets of tapes.

d.   Storage of backup data: Diskettes and tapes are stored in locked file cabinets in different offices and one set of the weekly backup diskettes is stored in a different building.

1.6.5   Statistical Analysis

By July 1995, we will have collected CVD mortality data for the population over an eleven and one-half year interval (1/1/84-6/30/95). Age and sex specific mortality rates will be calculated using person-years accrued from 1984 to last contact or death. The 1988 tribal roll will be updated to provide denominators as of 1993.

Cardiovascular disease mortality rates will be compared with those of the U.S., the states where the participants reside, and other populations. For the examined cohort, standardized mortality ratios (SMR) will be calculated if the number of deaths is large enough. A proportional mortality ratio will be calculated also.

Morbidity data obtained from surveillance activities will allow us to estimate 4-year incidence density (using person-years computed from baseline examination) for the examined cohort. Since the date of first event will be available, we will also be able to use survival data analysis techniques to identify risk factors. For example, the distributions of disease-free time for different "exposure" groups can be estimated by the Kaplan-Meier method and compared by a two-sample or K-sample test (5). To evaluate the risk factors simultaneously, Cox's proportional hazards model will be used in a stepwise manner to rank the variables according to their relative importance (5,6).

Morbidity data obtained from the clinical examination will be examined cross-sectionally and prospectively when combined with Phase I (baseline) data. Cross-sectional analyses will be performed using statistical methods similar to those described for the Phase I data. These include summary statistics for each variable, correlation analyses between variables, and comparisons of risk factors between disease groups. Prospective analyses will include the calculation of four-year incidence of CVD, PAD, diabetes and IGT, the association of baseline risk factors with CVD outcome at follow-up, and an examination of changes in risk factors. In addition, prevalence of rheumatic heart disease, left ventricular dysfunction and wall motor abnormalities and abnormal pulmonary function and their associations with risk factors will be examined.

The 4-year incidence density of CVD, PVD, hypertension, diabetes, IGT and hyperlipidemia will be calculated by sex using person-years of follow-up from time of baseline examination to diagnosis or last contact. The numerator will be the number of new cases that are diagnosed during the follow-up period and the denominator will be the total person-years of follow-up of subjects who were free of the disease under study at baseline. The incidence density so obtained can be compared to that obtained by using the surveillance data. This will provide a comparison between designs with an ongoing surveillance and with a single disease ascertainment at the end of the follow-up period.

For the association of baseline risk factors and changes in risk factors with disease outcome at follow-up, we will begin with univariate analysis. For continuous variables (or risk factors), summary statistics will first be calculated for each variable to provide a preliminary understanding of the variables. Internal comparisons of the means or distributions of baseline variable between the diseased and the non-diseased will be performed by t-tests or nonparametric tests. Appropriate transformations may be used to stabilize the variance.

For categorical variables, histograms and contingency tables will be used to present the data. Disease incidence rates will be compared between different baseline "exposure" groups by chi-square tests. For example, incidence rates of myocardial infarction will be compared between smokers and nonsmokers as indicated at baseline (i.e., Phase I). For dichotomous variables, relative risks and odds ratios will also be computed. Many continuous variables will be dichotomized or polychotomized so that relative risk and odds ratio can be estimated and contingency table analyses performed. For example, systolic blood pressure can be dichotomized into two groups: < 140 mmHg and ≥ 140 mmHg and total cholesterol values can be classified into three groups: < 200 mm/dl, 200-239 mm/dl, and ≥ 240 mm/dl. To adjust for the possible effect of confounding factors, stratified analysis will be performed using the Mantel-Haenszel method (5). In addition, the linear logistic regression analysis will be performed to assess the relative importance of the risk factors, both continuous and categorical. Adjusted odds ratios will be obtained.

Changes in risk factors over the follow-up period will be assessed and related to disease outcome. The changes may be categorical (e.g., from smokers to nonsmokers) or numerical (e.g., cholesterol value being 50 mg/dl lower). Similar statistical methods will be used to assess the association between changes and disease status at follow-up. Progression from IGT to diabetes and from normal glucose tolerance to IGT will be examined.

As in Phase I, we will analyze the risk factor data periodically during the clinical examination period for abstracts to be submitted to professional conferences. The risk factors will be examined for linear and nonlinear relationships, and interactions. Multiple regression analysis will also be performed to examine multiple variables simultaneously.

1.7   PUBLICATION POLICY

Overall responsibility for manuscript and abstract generation and approval for the Strong Heart Study lies with the Steering Committee, which also serves as the Publication Committee. This committee has developed procedures for generating manuscripts and abstracts as well as the formal requirements for manuscript approval prior to submission for publication or abstract submission before presentations.

The overall aim of this process is to encourage the preparation of manuscripts and abstracts while also providing appropriate control over their quality and content.

This section discusses the procedures for both the generation phase and the approval phase. It reviews the different types of possible publications and presentations, authorship, and general strategy for preparation of manuscripts and abstracts, and describes in more detail the requirements for each type of publication or presentation.

1.7.1   Types of Publications and Presentations

There are several types of publications and presentations for which approval procedures are established. These include:

(1)   Major descriptions of the design and conduct of the study.

(2)   Descriptions of results, based on data from all field centers, addressing the objectives of the study.

(3)   Descriptions of results based on data collected from a single field center.

(4)   Descriptions of methodological developments required to meet the needs of the study.

(5)   Articles to appear in proceedings of meetings for which no abstract was required.

(6)   Invited presentations.

(7)   The draft outline

(8)   Press releases or discussions with the media.

The Steering Committee is responsible for resolving any uncertainties as to which category a specific presentation or publication belongs.

1.7.2   Outline of the Preparation and Approval Process

The basic steps for the generation and approval of publications and presentations are listed below:

(1)   The Steering Committee designates a topic.

(2)   The Steering Committee selects a writing group and its chairperson. A member of the Coordinating Center will be included in each writing group.

(3)   The writing group prepares specifications for the manuscript and obtains Steering Committee approval.

(4)   The writing group prepares and communicates computational specifications to the Coordinating Center.

(5)   The Coordinating Center prepares statistical computations according to priorities specified by the Steering Committee.

(6)   The writing group prepares, reviews internally, and submits the completed document to the Steering Committee for review and approval.

(7)   The draft article is submitted to NHLBI, IHS and tribal groups for review and approval.

(8)   The manuscript is formally submitted to a journal or abstract selection process.

The overall responsibility for managing the entire process lies with the Steering Committee.

1.7.3   Authorship

The authorship policy varies according to the type of publication or presentation being considered. In all cases, the persons preparing the manuscript are listed as authors. Some abstracts and presentations can be listed as presented by someone for the study. The person assuming the primary responsibility will be listed as the first author. In addition, the phrase "Strong Heart Study" is to be included in the title and listed as a "keyword" whenever possible.

The Steering Committee is responsible for resolving any conflict or confusion that occurs with respect to appropriate recognition of authorship.

1.7.4 Manuscript and Abstract Generation

The general procedure for generating manuscripts or abstracts is for the Steering Committee to designate a writing group with the charge to develop the manuscript for publication or presentation. The impetus for this designation may come directly from the Steering Committee or may be in response to a request or suggestion from outside the committee. Once it is decided that a specific manuscript will be developed, the writing group and its chairperson will be specified.

Under normal circumstances the chairperson, who has the lead responsibility for this task, will also be listed as the first author. The chairperson also has the responsibility for listing the co-authors in the appropriate order. As indicated above, the Steering Committee serves as final arbitrator of any conflicts.

Individuals interested in preparing a manuscript or abstract on a specific topic must submit their proposal, which should include suggestions for writing group members, to the Steering Committee for approval. The proposal must include a clear statement of the nature of the publication, and should, if appropriate, also include the hypotheses to be addressed and the types of statistical computations or data summarizations likely to be required.

The Steering Committee has the responsibility for reviewing these proposals, both for appropriateness and for a priority designation. The Steering Committee also ensures that the different participating centers and groups are appropriately represented and that appropriate recognition is provided.

Once the specifications for the manuscript have been approved, the requirements for statistical computing can be formally communicated to the Coordinating Center. Requests will be processed according to the priorities specified by the Steering Committee. The Coordinating Center has representation on the writing group whenever possible and this person serves as the liaison to the writing group both for communications about computing issues and for providing or obtaining appropriate statistical input.

The Steering Committee reviews the progress that each writing group is making toward the completion of its task and makes those changes required for the timely completion of each manuscript or abstract.

1.7.5 Approval Procedures

A manuscript stemming from the Strong Heart Study is submitted to the chairperson of the Steering Committee, who sends copies of the manuscript to all Steering Committee members for their critique. Upon receiving the critiques, two courses of action are possible: (1) If the chairperson deems the reviewers suggestions to be mainly editorial in nature, she may approve the manuscript and request that the authors incorporate

suggested changes to the final version, or submit in writing reasons for not doing so. No further action is needed from the Steering Committee; or (2) If, in the chairperson's judgment, critiques entail substantive changes, the revised manuscript must be further reviewed by the primary reviewers. Approval by NHLBI, IHS and tribes will next be initiated. Each center will be responsible for obtaining local IHS and tribal approval. The Dakotas Center, on behalf of the three centers, will also submit the manuscript to IHS headquarters for approval of the manuscript.

1.7.6   Press Releases and Media Discussions

In general, scientific findings from the Study made available to the media will involve those findings being presented at scientific meetings and being published in the scientific literature. Such presentations and publications require prior clearance as noted above. In some circumstances, media discussions and press releases may be appropriate to clarify scientific findings for the lay public, but they should not be used as forums to release new information. Investigators are requested to keep the Program Office informed of contacts with representatives of the major national media and of major national media coverage of information which they have supplied. If a situation arises in which it appears desirable to release to the media new information not otherwise cleared for presentation or publication, prior clearance from both the Steering Committee and the Program Office is required.

Release of general descriptive information about the study for local use (such as a local newspaper, university newsletter or state medical society journal) does not require prior approval. Use of centrally prepared materials for such purposes is encouraged. A copy of any resultant article should be sent to the Program Office and the participating tribes. All those communicating with the media will be sensitive of the special needs and concerns of the Indian Communities involved. Any interviews or photographs involving tribal members must have prior approval of the tribe.

1.8.   Ancillary Studies Policy

1.8.1   General Policy

To enhance the value of Strong Heart Study and to ensure the continued interest of the investigators, the Steering Committee welcomes proposals from individual investigators to carry out ancillary studies. Nevertheless, to protect the integrity of the Study, such ancillary studies must be reviewed and approved by the Steering Committee before their inception. In general, ancillary studies require outside (non-Strong Heart Study) funding.

1.8.2   Definition of an Ancillary Study

An ancillary study is one based on information from the Study participants in an investigation which is not described in the Strong Heart Study protocol and involves data which are not collected as part of the routine Strong Heart Study data set. The core Strong Heart Study includes the use of blood and DNA stored for case-control studies selected by the Steering Committee; these are not considered ancillary studies.

1.8.3   Requirements for Approval of an Ancillary Study

Before an ancillary study can be approved, it must be shown that the ancillary study will have scientific merit but will not do any of the following:

(1)   Interfere with the completion of the main objectives of the Strong Heart Study.
(2)   Adversely affect participant cooperation in compliance in the Strong Heart Study.
(3)   Create a serious diversion of study resources (personnel, equipment or study samples), either locally or centrally.
(4)   Jeopardize the public image of the Strong Heart Study.

1.8.4   Preparation of Request for Approval of an Ancillary Study

A written request for approval of an ancillary study should be submitted to the Steering Committee and should contain the following information:

(1)   Description of objectives.
(2)   Scientific merit of study.
(3)   Methodology for data collection.
(4)   Proposed statistical analyses.
(5)   Names of definite or possible collaborators.
(6)   Proposed funding sources.
(7)   Discussion of impact on main Strong Heart Study.

1.8.5   Review of Ancillary Study Proposals

The Steering Committee will review and will approve, reject or request modification of ancillary study proposals in a timely manner. At least one Strong Heart Study investigator must be included as a co-investigator in each proposal. Strong Heart Study investigators other than those submitting the proposal may request to become collaborators on a proposal if they have a specific interest in the topic. The key criteria for approval of proposals are scientific merit and impact on the main Study. Formal IRB approval will be required if such studies require interviews or additional procedures of the participants. The principal investigator of the ancillary study is responsible for obtaining IRB approval from the 3 areas and the national IHS IRBs.

If the proposal will initialize laboratory specimen and data previously collected or routinely collected as part of SHS to answer research questions related to cardiovascular and pulmonary diseases, the IRBs will be informed of the changes in protocol with the annual IRB report. If the steering committee feels that the ancillary study will result in a major change in the protocol, the principal investigator will be required to seek IRB approval prior to conducting the study. Any ancillary study that is not related to cardiovascular or pulmonary diseases will require IRB approval.

1.8.6   Analysis and Publication of Results of Ancillary Studies

The investigator of the ancillary study, and if necessary the Steering Committee, will consult with the Coordinating Center during data analysis to ensure that all study data used in analysis of ancillary study results are consistent with data in the main study database. Ancillary study investigation will be required to sign a confidentiality statement (Appendix 6). In addition the investigation will need to sign a statement that indicates his/her willingness to submit draft manuscripts for approval by the Steering Committee, NHLBI, IHS and the tribes. Manuscripts resulting from ancillary studies will require approval by the Steering Committee and by NHLBI, IHS and the tribes prior to submission for publication or presentation. The investigator who assumes lead responsibility for the ancillary study shall be listed as senior author. The phrase "The Strong Heart Study" should be included in the title and listed as a key word whenever possible. Manuscripts will also contain an appendix listing all Strong Heart Study Principal Investigators as well as other individuals deemed appropriate.

1.8.7 Agreement for Ancillary and Collaborative Investigation

The following agreement must be signed by ancillary and collaborative investigators:

I agree to read and follow the SHS protocol with regard to analysis of Strong Heart Study data I request. I will comply with the SHS policies regarding maintaining data security and will sign a confidentiality statement. I have attached a research protocol describing how I will use these data to better understand cardiovascular and pulmonary diseases in American Indians and how to benefit the health of American Indians.

I agree to submitting a draft report of the results of this analysis for review and approval of the SHS Steering Committee, NHLBI, IHS and the participating tribes. If approval for publication is not granted, I agree not to publish these results.

I understand the SHS Steering Committee will assist me in revising my report in such a way that will make it acceptable for publication. I agree to include one of the SHS Steering Committee members as a co-investigator and a co-author.

Signed:_____   Date:_____

1.8.8 Feedback of Results of Ancillary Studies to Participants

Results of ancillary studies shall be reported to participants and/or their physicians if medically useful. Such reporting should follow standard Strong Heart protocol for notification of participants. A copy of any resultant article should be sent to the Program Office and the participating tribes.

2. Mortality Survey

2.1 ELIGIBILITY CRITERIA

Fatal events are selected according to the following eligibility criteria:

(1)   Age. Only deaths at ages 35 to 74 and participants of SHS-I are included.

(2)   Tribal Affiliation. The decedent must have been enrolled in one of study tribes.

(3)   Place of Residence. The decedent must have lived within the study community. The residence recorded on the death certificate determines eligibility. People institutionalized at the time of death will be included.

(4)   Time. Only deaths occurring between January 1, 1989 and December 31, 1994 are eligible.

2.2 DEFINITIONS OF CVD DEATHS

The following will be the primary events of interest:

(1)   Definite fatal myocardial infarction (MI)

(2)   Definite sudden death due to coronary heart disease (CHD)

(3)   Definite fatal CHD

(4)   Possible fatal CHD

(5)   Definite fatal Stroke

(6)   Possible fatal stroke

(7)   Definite fatal congestive heart failure (CHF)

(8)   Possible fatal CHF

(9)   Other fatal CVD

All death certificates will be coded by the study nosologist. After coding the following ICD codes will be utilized to identify subjects for detailed mortality review: possible cardiovascular disease 250, 390-448, 518.4, 585, 798 and 799.

Criteria used for defining the primary CVD deaths are the International Diagnostic criteria for acute myocardial infarction and acute stroke (7) and criteria for fatal CHF of the Framingham study (8).

2.2.1 Definite fatal myocardial infarction (MI)

1a   Definite MI within 4 weeks of death by criteria:

1. Evolving diagnostic ECG

    AND/OR

2. Diagnostic ECG and abnormal enzymes

    AND/OR

3. Prolonged cardiac pain and abnormal enzymes.

    OR

1b Acute MI diagnosed by autopsy

    AND

2   No known nonatherosclerotic or noncardiac-atherosclerotic process that was probably lethal according to death certificate, autopsy report, hospital records, or physician records.

2.2.2 Definite sudden death due to coronary heart disease (CHD)

1   Death witnessed as occurring within 1 hour after the onset of severe cardiac symptoms (cardiac pain - see below, shortness of breath, fainting) or within 1 hour after the subject was last seen without symptoms

    AND

2   No documentation of definite acute MI within 4 weeks prior to death by criteria (see 1a in Section 2.2.1 for criteria for definite MI)

    AND

3   No known nonatherosclerotic or noncardiac-atherosclerotic process that was probably lethal according to death certificate, autopsy report, hospital records, or physician report.

2.2.3   Definite fatal CHD

1   Death certificate with consistent underlying or immediate cause(s) (ICD-9 codes 410-414)

   AND

2   No documentation by criteria of definite acute MI within 4 weeks prior to death

   AND

3   Criteria for sudden death not met

   AND

4   No known nonatherosclerotic or noncardiac-atherosclerotic process or event that was probably lethal according to death certificate, autopsy report, hospital records, or physician records

   AND

5a   Previous history of MI according to relative, physician, or hospital records, or definite MI (see criteria above) or possible MI by criteria below:

   One or more of the following categories: *

   1)   Equivocal enzymes and equivocal ECG (with or without pain)
   2)   Equivocal enzymes and diagnostic ECG (no pain)
   3)   Abnormal enzymes and other ECG (no pain)
   4)   Abnormal enzymes and equivocal ECG (no pain)
   5)   Abnormal enzymes alone (no pain, ECG absent or uncodeable)
   6)   Prolonged cardiac pain and equivocal enzymes (ECG absent or uncodeable)
   7)   Prolonged cardiac pain and equivocal ECG (enzymes incomplete)
   8)   Prolonged cardiac pain and diagnostic ECG (equivocal or incomplete enzymes)
   9)   Prolonged cardiac pain alone (ECG and enzymes incomplete)
   10) Prolonged cardiac pain, "other" ECG, equivocal enzymes
   11) Prolonged cardiac pain, "other" ECG, incomplete enzymes

   OR

5b   Autopsy reporting severe atherosclerotic-coronary artery disease or old MI without acute MI (50% proximal narrowing of two major vessels or 75% proximal narrowing of one more vessel if anatomic details given)

OR

5c   Rapid death:

Death occurring greater than 1 and less than or equal to 24 hours after the onset of severe cardiac symptoms or after subject was last seen without symptoms.

*   Definitions are given in Section 2.3.

2.2.4 Possible fatal CHD

1   No documentation by criteria of definite acute MI within 4 weeks prior to death

AND

2   No documentation by criteria of definite sudden death

AND

3   No documentation by criteria of definite fatal CHD

AND

4   Death certificate with consistent underlying or immediate cause (ICD-9 codes 410-414)

AND

5   No known nonatherosclerotic or noncardiac-atherosclerotic process that was probably lethal according to death certificate, autopsy report, hospital records, or physician records.

2.2.5 Definite Fatal Stroke

    1a   Cerebral infarction or hemorrhage diagnosed at autopsy

                AND

    1b   No other disease process or event such as brain tumor, subdural hematoma, subarachnoid hemorrhage, metabolic disorder, or peripheral lesion that could cause localizing neurologic deficit or coma - according to death certificate, autopsy, hospital records, or physician records

                OR

    2a   History of rapid onset (approximately 48 hours from onset to time of admission or maximum acute neurologic deficit) of localizing neurologic deficit and/or change in state of consciousness

                AND

    2b   Documentation of localizing neurologic deficit by unequivocal physician or laboratory finding within 6 weeks of death with >24 hours duration of objective physician findings

                AND

    2c   See list under (1b) above.

2.2.6 Possible Fatal Stroke

    1   Death certificate with consistent underlying or immediate cause (ICD-9 codes 431-437)

                AND

    2   No evidence at autopsy examination of the brain, if performed, of any disease process other than cerebral infarction or hemorrhage that could cause localizing neurologic signs (see (1b) above).

2.2.7 Definite Fatal CHF

Two major or one major and 2 minor criteria must be present concurrently.

<u>Major criteria</u>

    Paroxysmal nocturnal dyspnea or orthopnea
    Neck vein distention
    Rales
    Cardiomegaly
    Acute pulmonary edema
    S_3 gallop
    Increased venous pressure > 16 cm water
    Circulation time $\geq$ 25 seconds
    Hepatojugular reflux

<u>Minor criteria</u>

    Ankle edema
    Night cough
    Dyspnea on exertion
    Hepatomegaly
    Pleural effusion
    Vital capacity reduced by one-third from predicted
    Tachycardia (rate of $\geq$ 120/min)

<u>Major or Minor criterion</u>

    Weight loss $\geq$ 4.5 kg in 5 days in response to treatment. No know noncardiac process leading to massive fluid overload such as renal failure.

2.2.8 Possible Fatal CHF

    Death certificate with consistent underlying or immediate cause, but neither autopsy evidence nor adequate pre-terminal documentation of the event.

2.2.9 Other Fatal CVD

    1.   Definite other fatal CVD

        1a)   Autopsy evidence consistent with other CVD as cause of death

               OR

        1b)   Death certificate with consistent underlying or immediate cause

               AND

        2)   Adequate documentation in medical records

    2.   Possible other fatal CVD

        Death certificate with consistent underlying or immediate cause, but does not satisfy any of the above criteria.

2.3   DEFINITION OF ABNORMAL ECG, ABNORMAL ENZYMES AND PROLONGED CHEST PAIN

2.3.1 Abnormal ECG

    1.   Evolving Diagnostic ECG

        An evolving pattern on serial ECGs of a diagnostic ECG. (An evolving pattern of changes [appearance or disappearance within lead groups: anterior (V_1 -V_5); lateral (I, aV_L, V_6); or inferior (II, III, aV_F)] establishes the infarct as acute. Two or more ECG recordings during the hospitalization are needed for this classification.)

        To Qualify as a Q wave, deflection should be at least 0.1 mV (1 mm.) in amplitude. Possibilities:

        a.   No Q wave in one ECG record followed by a record with a diagnostic Q wave.

               OR

        b.   An equivocal Q wave and no major ST segment depression in one ECG followed by a record with a diagnostic Q wave PLUS a major ST segment depression.

OR

c. An equivocal Q wave and no ST segment elevation in one ECG record followed by a record with a diagnostic Q wave PLUS ST segment elevation > 1 mm.

OR

d. An equivocal Q wave and no major T wave inversion in one ECG record followed by a record with a diagnostic Q wave PLUS a major T wave inversion.

OR

e. No Q wave and no ST Junction depression $\geq$ 0.5 mm. and flat or downsloping ST segment depression followed by a record with an equivocal Q wave PLUS ST Junction and flat or downsloping ST depression of 0.5 mm.

OR

f. No Q wave and no ST elevation > 1 mm. followed by a record with an equivocal Q wave PLUS ST elevation > 1 mm.

OR

g. No Q wave and no T wave findings diagnostic of infarction followed by a record with an equivocal Q wave PLUS T wave findings diagnostic of infarction.

2. DIAGNOSTIC ECG WITH Q WAVE

a. Diagnostic Q and QS patterns.

3. DIAGNOSTIC ECG WITHOUT Q WAVE

a. ST segment elevation PLUS T wave depression indicative of infarction. (T wave depression cannot be used in the presence of ventricular conduction defects.)

4. EQUIVOCAL ECG WITH Q WAVE

    a. ECG with Q and QS pattern possibly representing infarction.

5. EQUIVOCAL ECG WITHOUT Q WAVE

    a. ST junction (J) and segment depression or T wave inversions or ST segment elevations possibly representing infarction.

6. OTHER

    a. All other findings, including normal.

7. UNCODEABLE ECG

    a. Missing Leads
    b. Baseline drift (1 in 20) if it obscures ST-T segment.
    c. Muscle tremor giving 2 mm. peak-to-peak oscillation.
    d. Other technical errors making Q wave measurements impossible.
    e. Major abnormal QRS conduction patterns(BBB, pacer, etc.)

2.3.2 Abnormal Enzyme

To be able to be used to evaluate an MI, enzymes must have been measured within 1-4 days of admission or onset of acute event, whichever is later.

1. Abnormal Cardiac Enzymes

    Enzymes are classed as "abnormal" if any enzyme values recorded meet any of the following criteria:

    1a) CK-MB is "present" (if laboratory uses the criterion of "present" or "absent" without reporting a more specific value) or the CK-MB (heart fraction) is at least twice the upper limits of normal (if hospital uses quantitative criteria) or 10% of the total CK value, and total CK is at least twice the upper limit of normal.

        AND

    1b) There is no known non-ischemic cause (cardiac surgery, severe muscle trauma, rhabdomyolysis) for the elevated enzyme value.

OR

2a)   The ratio $LDH_1 : LDH_2 > 1$

        AND

2b)   There is no evidence of hemolytic disease.

        OR

3a)   Total CK and LDH are both at least twice the upper limits of normal. (These increases do not have to occur on the same day.)

        AND

3b)   There is no known non-ischemic cause (cardiac surgery, severe muscle trauma, rhabdomyolysis) for the elevated enzyme value and no evidence of hemolytic disease.

2.   Equivocal Cardiac Enzyme

        Enzymes are classed as "equivocal" if the criteria for abnormal enzymes are not met and if:

1)   Either total CK or total LDH are at least twice the upper limits of normal.

        OR

2)   Both total CK and total LDH are between the upper limits of normal and twice the upper limits of normal. (These increases do not have to occur on the same day.)

        OR

3)   CK-MB= 5-9% of total CK or is "weakly present".

        A summary of the enzyme diagnostic criteria, as related to total CK and LDH is given in the following algorithm:

		Equivocal	Equivocal	**Abnormal**
TOTAL LDH	Twice Upper Limit of Normal			
	Upper Limit of Normal	Normal	Equivocal	Equivocal
	Normal	Normal	Normal	Equivocal
		Normal	Upper Limit of Normal	Twice Upper Limit of Normal

TOTAL CK

2.3.3 Prolonged Cardiac Pain

Pain having the following characteristics: Occurring anywhere in the anterior chest, left arm or jaw, which may also involve the back, shoulder, right arm, or abdomen on one or both sides and lasting for more than 20 minutes.

2.4 IDENTIFICATION AND CONFIRMATION OF CVD DEATHS

Fatal events will continue to be identified and investigated during Phase II using the same protocol as was used in Phase I. Procedures for abstracting and coding death certificates and for the mortality review are described in detail on pages 22-34 and 149-203 in the Strong Heart Study (SHS) Phase I manual. In Phase II, all deaths occurring in the population during the calendar years 1989 through 1994 will be identified. Additional information will be obtained for those death certificates with any mention of cardiovascular disease. These data will be reviewed for assignment of the underlying cause of death.

The examination cohort will be monitored in an on-going fashion to identify deaths. The following sources will be monitored on a regular basis to identify additional deaths in the cohort as they occur: local newspapers and community notices, community and tribal members, IHS, tribal and BIA records. Near the end of 1995, the final year of data acquisition in Phase II, the State Health Departments will be contacted to identify death certificates in the study communities for those deaths that may have been missed using other sources.

2.4.1 Procedure

The identification and confirmation of CVD deaths will involve the following steps: (1) identification of all deaths occurring in the eligible population during 1989-94, (2) obtaining all death certificates, (3) coding of all death certificates by the central nosologist, (4) identification of potential CVD deaths, (5) obtaining Coroner's/Medical Examiner's report, (6) review autopsy reports, (7) chart review, and (8) independent confirmation of CVD deaths by the Mortality/Morbidity Review Committee comprised of Dr. Maurice Sievers, Dr. Wm. James Howard, and Dr. Arvo Oopik.

STEP 1:   Identification of all deaths

All deaths that satisfy the Eligibility Criteria (1) - (4) in Section 2.1 will be identified by each center from tribal records, IHS hospitals, BIA, State Department of Health and/or the National Death Index. The name, date of birth, date of death and place of death will be obtained for each eligible death. Persons who died out-of-state when visiting other states will be included.

STEP 2:   Obtaining death certificates and reviewing charts

With the names of the decedents, dates of birth, dates of death, and places of death, copies of death certificates of all deaths will be obtained from the State Department of Health. The Death Certificate Form (Appendix 7) will be completed by the local data abstractor and mailed to the Coordinating Center.

STEP 3:   Coding of death certificates by central nosologist

The local center will stamp the back of the death certificate, add the ID number immediately above the stamp and send only the death certificate to the central nosologist,

> Mr. Karl E. Wise
> 36 Fox Grape Lane,
> Southern Shores
> Kitty Hawk, NC 27949

for coding of the cause of death. The corresponding Death Certificate Forms will simultaneously be sent to the Coordinating Center. Mr. Wise will, in a standardized approach using ICD 9th Revision, record the codes on the back of the death certificate and return it to the Coordinating Center. The nosologist's codes will be entered into the computer. A copy of the codes will be sent to the Study Center by the Coordinating Center.

STEP 4:   Identification of potential CVD deaths

Potential CVD deaths will be identified by each Study Center after receiving the codes from the Coordinating Center.

A potential CVD death is defined as having mention of any of the following anywhere on the death certificate.

Any type of cardiovascular disease
Diabetes
Acute edema of lung
Renal disease
Sudden death, cause unknown

If there is any question as to whether a death should be considered a potential CVD death, the P.I. should be consulted.

The following steps are for the potential CVD deaths only.

STEP 5:   Obtaining Coroner's/Medical Examiner's Report

If it is indicated on the death certificate that an autopsy was performed, the Coroner's/Medical Examiner's Report will be obtained by each study center. Photocopy the autopsy report, complete the Mortality Medical Records Abstract Form, attach both to the death certificate, and send the entire package, including Final Decision Form, to Dr. Sievers for confirmation.

STEP 6:

Review medical chart to see if the decedent was hospitalized within one year prior to death and fill out Mortality Survey Medical Records Abstract Form (Appendix 9) in order to identify possible CVD events between 1989 and 1994. The Chart Request Form in Appendix 16 can be used to record charts needed from each involved hospital. Note that if the decedent was a participant in phase I exam, all hospital admissions between exam and death must be reviewed.

STEP 7:   Confirmation of CVD deaths

a.   If the decedent was hospitalized within one year prior to death, the Mortality Survey Medical Records Abstract Form will be completed. The Medical Records Abstract Form, the death certificate and the Coroner's/Medical Examiner's report, if available, will be sent to Dr. Sievers for confirmation.

b.   If the decedent died prior to arrival at the hospital, upon arrival, or in any other non-hospital location (e.g., home, nursing home), and if available information is not sufficient to determine whether the death was due to a cardiovascular problem, the attending physician, and an informant will be identified from the death certificate or other sources and contacted for an interview. The Physician's Questionnaire (Appendix 10), Informant Interview Form (Appendix 11), and the Medical Records Abstract Form will be completed. These three forms as well as the death certificate and coroner's/medical examiner's report (if available) will be forwarded to Dr. Sievers. A Final Decision Form (Appendix 12) will also be mailed to Dr. Sievers from the study center for recording his final decision. The study center will stamp the ID number and fill out the patient's name on the Final Decision Form for Dr. Sievers. Dr. Sievers will return the completed Final Decision Form to the Coordinating Center for data entry. The Coordinating Center will forward a copy of the Decision Form to the Study Center. For any equivocal cases Dr. Sievers will forward all information to Dr. Wm. James Howard for independent classification. In addition, Dr. Wm. James Howard will independently reclassify 33 percent of deaths, and other will independently reclassify the rest 67 percent.

A flowchart describing the procedure outlined above and a checklist which should be followed to assure that all steps are completed are given in Appendices 13 and 14, respectively.

2.4.2   Review of Medical Charts of the Decedents

Unless the Coroner's report is conclusive, medical records of the decedent will be reviewed and pertinent data abstracted and photocopied using the Medical Records Abstract Form. For deaths that occurred in hospitals other than IHS hospitals, additional efforts will be made to secure medical information. If the patient was hospitalized in more than one facility without intervening discharge, all available medical records will be reviewed to complete the Medical Record Abstract Form. Discharge summaries, ECGs, X-ray reports, etc. will be photocopied and attached to the Form. If the patient died in a hospital as an in-patient, data accumulated in the period of hospitalization will be reviewed and abstracted. If the patient died out-of-hospital or died upon arrival at the hospital, available information in the medical records for relevant hospitalizations and outpatient visits within one year prior to death will be reviewed and abstracted.

2.4.3   Informant Interview

Using name and address information from the death certificate, an attempt will first be made to contact and interview the spouse or a first-degree relative (i.e., son, daughter, or sibling) of the decedent, or someone else who witnessed the death. The following

procedure will be followed:

(1)   Find the informant's telephone number and/or address.

(2)   If the telephone number is available, call him/her to request permission to interview and to set up an interview appointment. The interview may be conducted over the telephone, or if necessary, in person using the Informant Interview Form.

(3)   If phone contact is not possible, the local community health representative or public health nurse will be asked to assist in arranging the interview.

(4)   If the informant cannot be contacted by phone or in person, a form letter, a reply letter and a self-addressed and stamped envelope (Appendix 15 a and b) will be sent asking the informant for permission for an interview and convenient time for the interview. If the form letter is sent and no reply is received in three weeks, another such letter is sent by certified mail. If no reply is received within one month, no further effort to contact the individual is made.

When the death is witnessed by someone other than a member of the decedent's family, both a family member and the witness are interviewed. In such a case, the information from both interviews are recorded on separate Informant Interview Forms. Up to three (the three best) Informant Interview Forms may be completed for a given event.

2.4.4   Death Occurring Outside the Study Community

Eligible deaths outside of the study area, but within the State, will be included in the above review and confirmation procedure. For eligible out-of-state deaths, we will attempt to obtain an abstract or summary from the hospitals where they died and interview an informant. Their local medical charts will also be reviewed.

3.   Morbidity Survey

3.1   ELIGIBLE POPULATION

Only those persons who participated in the Phase I physical examination will be followed for incident events of cardiovascular disease in Phase II. Because the interval between the Phase I and Phase II examinations is relatively short (maximum, 6 years; minimum 1.5 years; mean 4 years), the major point at which the occurrence of new events will be ascertained will be at the Phase II examination. Determining the incidence of events at this time will allow for more thorough collection of data and for obtaining signed consent for review of medical records pertaining to the events of interest. In Phase II, the following incident events will be identified: myocardial infarction, stroke, congestive heart failure, angina and peripheral vascular disease. The occurrence of coronary bypass surgery, angioplasty or similar procedures will also be determined. Prevalence of the following conditions/indicators will also be ascertained at the Phase II exam: valvular heart disease, positive cardiac catheterization, positive treadmill test, left ventricular hypertrophy, other left ventricular dysfunction, cardiac wall motion abnormalities, and obstructive lung disease. The same definitions and criteria for these events used in Phase I will be used in Phase II.

3.2   SURVEILLANCE EVENTS

Two types of frequency measures will be used, total incidence and incidence rate. All cases of MI and stroke, as well as new cases (first occurrence), occurring during the study interval will be ascertained. Data obtained from review of medical records will be used to calculate total incidence and incidence rates of acute myocardial infarction and stroke. Only information for those events with discharge diagnoses between 1989 and 1995 and for participants in the Strong Heart Study Phase I examination will be abstracted. The following types of CVD will be ascertained:

1.   Acute Myocardial Infarction (ICD-9 code 410)

2.   Stroke (ICD-9 codes 431-432, 434, 436)

3.   Coronary Heart Disease

4.   All other Cardiovascular Disease

3.3   DIAGNOSTIC CRITERIA - NON-FATAL CARDIOVASCULAR DISEASE

1.   Definite Myocardial Infarction (MI)

Minnesota codes 1.1.x or 1.2.x except 1.26. and 1.28 with no 7.1 or 7.4
History of MI verified by chart review as definite MI

2.   Possible Myocardial Infarction

Minnesota codes 1.3.x, 1.2.6, or 1.2.8 with no 7.1 or 7.4
History of MI verified by chart review as possible MI

3.   Definite Coronary Heart Disease (CHD)

Definite MI, or
Definite CHD verified by chart review to include cardiac cath,
        proven coronary artery disease, PTCA, coronary artery bypass grafting, or
        abnormal stress ECG plus abnormal imaging (i.e., both must be abnormal), or
Angina Pectoris plus   LBBB (7.1.1) or
                        ST changes (4.1) or
                        T wave changes (5.1), or
                        verified possible MI.

4.   Possible Coronary Heart Disease

Possible MI by ECG (1.3.x, 1.2.6, 1.2.8), or verified by chart review, or
Minnesota codes in one of the following 7.1, 4.1, 4.2, 5.1, 5.2, 7.4, or
Positive functional test of ischemia (such as treadmill) without invasive confirmation, or
Possible ECG or imaging in scintigraphic studies (not both), or
Unconfirmed self reported history of MI, or
Self reported Angina Pectoris.

5.   Other Non-fatal Cardiovascular Disease

Any CHD
Congestive Heart Failure
Cardiomyopathy
Valvular Heart Disease
Left ventricular Hypertrophy by Echocardiogram
Left ventricular Hypertrophy by ECG (3.1 or 3.3 plus 4.1-4.3 or 5.1-5.3)
Ankle Arm Index <= 0.8
Atrial Fibrillation
Minnesota codes 4.1, 5.1, 6.1, 6.2, 6.8, 7.1, 7.2, 7.4

Noncoronary heart surgery or carotid or other vascular surgery
Pacemaker implantation
Bruits by physical examination
Intermittent Claudication by Rose Questionnaire
Positive non-coronary angiography

3.4 PROCEDURE FOR IDENTIFICATION OF INCIDENT AND RECURRENT CASES

Data on the incidence and prevalence of endpoints of interest will be obtained primarily at the time of the Phase II examination. Other sources of this information include: IHS user listings and discharge records of other community hospitals. Relevant information will be collected from the medical record for each documented event to allow independent confirmation of the diagnosis of a case.

STEP 1:   Identification of potentially eligible cases.

Participants at the clinical examination will be asked if they had an MI, stroke or any other heart or circulation problems during 1989-95. Positive answers will be confirmed by chart review. When reviewing IHS user lists or hospital discharge listings, names, chart numbers and other relevant information for pulling charts for review should be recorded on the Chart Request Form (Appendix 16). The following ICD-9 codes are associated of events of interest.

1.   MYOCARDIAL INFARCTION (ICD-9 codes 402, 410-414, 427-428, 518.4)

   402   Hypertensive heart disease
   410   Acute myocardial infarction
   411   Other acute and subacute forms of ischemic heart disease
   411.0   Postmyocardial infarction syndrome
   411.1   Intermediate coronary syndrome
   411.2   Other - includes coronary insufficiency (acute), microinfarct of heart, subendocardial ischemia
   412   Old myocardial infarction
   413   Angina pectoris
   414   Other chronic ischemic heart disease
   427   Cardiac dysrhythmia
   428   Heart failure
   428.0   Congestive heart failure
   428.1   Left heart failure
   428.9   Heart failure, unspecified
   518.4   Acute edema of lung, unspecified

2.   CEREBROVASCULAR DISEASE (ICD-9 430-438)

430   Subarachnoid hemorrhage
431   Intracerebral hemorrhage
432   Other and unspecified intracranial hemorrhage
433   Occlusion and stenosis of precerebral arteries - includes embolism, narrowing, obstruction or thrombosis of basilar,carotid, and vertebral arteries
434   Occlusion of cerebral arteries
435   Transient cerebral ischemia
436   Acute, but ill-defined, cerebrovascular disease - includes CVA NOS, Stroke
437   Other and ill-defined cerebrovascular disease - includes cerebral atherosclerosis, chronic cerebral ischemia, hypertensive encephalopathy, cerebrovascular disease or lesion not otherwise specified.
438   Late effects of cerebrovascular disease

STEP 2:   Review of medical record for eligibility

If the examination participant reports a history of MI, stroke or diagnostic procedures or treatments of interest between 1989 and re-examination, they will be asked at which hospital they were cared for so that records can be obtained. Release of clinical information forms will be obtained for all non-IHS facilities, if required by local Institutional Review Board (IRB) and the standard IHS Authorization For Release of Information may be used.

If required by the local IRB, consent for release of clinical information will be obtained from the participant or the next-of-kin before any charts are reviewed.

STEP 3:   Confirmation of event occurrence and incident status.

Because discharge diagnoses may be improperly recorded and a variety of associated codes will be screened, it is important to confirm that one of the events of interest has, in fact, occurred. Information in the record pertaining to the admission by which the potential case was identified (the index admission) should be reviewed. Check the discharge diagnoses listed on the face sheet of the admission and read the discharge summary.

Myocardial infarction and stroke are defined as "new" if the participants gave a negative history during the Phase I examination and there was no mention in the medical record of a previous episode. An event is "new" only if it first occurred after the Phase I examination. All events of interest occurring during the study interval should be abstracted.

STEP 4:   Medical record abstract for incident cases.

If the index admission is for one of the study events (whether or not it is the first occurrence), an appropriate medical record abstract form for that admission should be completed (Appendix 17(a)). If evidence is present suggesting that one or more myocardial infarctions or strokes occurred, a separate chart abstract form will be completed for each event. Separate events must have a 28 day period when the patient is discharged from an acute care facility after a previous event. (If the participant is a study death, the abstract of medical records for decedents should also be completed.) If the medical record is not eligible for abstraction, the reason for exclusion (i.e., event occurred outside of the calendar years of the study, not a study event) should be noted on the front on the SHMORB.

Photocopy checklists for MI, stroke, and/or cardiovascular procedures and tests should be completed, depending on the material that is being collected. High resolution photocopies of ECGs taken as evidence of a myocardial infarction (see Section 3.3.3) should be arranged in chronological order from earliest to latest. ECG series for each case will be sent to the ECG Reading Center (Fitzsimons) with a completed Possible Myocardial Infarction ECG Analysis Field Sheet (Appendix 17(b)) and a blank ECG Center Sheet (Appendix 17(c)) with ID number stamped. The ECG series will be reviewed independently by three cardiologists and results recorded on the ECG Center Sheet which will then be returned to the Coordinating Center.

The Morbidity Survey Medical Records Abstract form, Photocopy Checklists, the ECG Analysis Field Sheet, the ECG Analysis ECG Center Sheet and the Morbidity Survey Decision form (Appendix 17(d)) will be sent by the Coordinating Center to Dr. Arvo Oopik for confirmation. Dr. Oopik will return the entire package with the completed Decision form to the Coordinating Center. For any equivocal cases Dr. Oopik will forward all information to Dr. Wm. James Howard for independent confirmation. In addition, Dr. Wm. James Howard will independently reclassify a random ten percent of cases.

Table 1. Endpoints

Endpoints/Events	Type of Rate	Source of Data
Primary Clinical Endpoints		
Myocardial Infarction	I	S, E
Stroke	I	S, E
Congestive Heart Failure	I	S, E
ECG evidence of new MI	I	E
Coronary bypass surgery/angioplasty	I	S, E*
Secondary Events of Interest/Pre-clinical Disease		
Valvular Heart Disease	P	E
Angina	I	E
Peripheral Vascular Disease	I	E
Cardiac catheterization, positive	P	S, E*
Positive treadmill test	P	S, E*
Left ventricular hypertrophy (LVH)	P	E
Global evaluation of LV function	P	E
Cardiac wall motion abnormalities	P	E
Obstructive lung disease (Ratio FEV1/FVC or FEV1/SVC)	P	E

I = Incidence   P = Prevalence
S = Surveillance contact   E = Examination, Phase II
E* = By interview, with medical record confirmation.

For each event, there is a designation as to whether it is an incident or prevalent event and the source(s) through which it will be initially ascertained. Because baseline data for the primary endpoints are available from Phase I, new events ascertained in Phase II will be incident events, and all of the primary endpoints, with the exception of ECG evidence of new myocardial infarction, can be identified both through surveillance contacts and during the Phase II examination. The majority of secondary events of interest shown in the table were not specifically ascertained in Phase I, and thus, persons identified with these conditions in Phase II will be prevalent cases. In addition, most of the secondary events will be ascertained only through systematic, uniform examination of participants in Phase II.

4. Procedures for Training & Quality Control
of Mortality & Morbidity Surveillance

4.1 TRAINING

Interviewers and data abstractors will be centrally trained at the July 1993 training meeting in South Dakota. Training will include instructions in reviewing and abstracting of charts and instructions in transcribing of information on death certificates and medical examiner reports. Training will include:

1. Adherence to the standardized protocol
2. Techniques for locating information in the charts
3. Dealing with problems encountered in the charts
4. Post-abstraction responsibility for the data

The training sessions will consist of:

1. Explanation of the procedure for abstracting
2. Demonstration by the instructor of abstraction procedures
3. Performance of abstraction by the trainee with instructor observing
4. Abstraction of records by both the trainee and the instructor with verification for completeness, consistency and accuracy

4.2 QUALITY CONTROL

4.2.1 Ascertainment of Cause of Death

In the mortality study, duplicated records will be sent to the Mortality Review Committee by each center. Each reviewer will independently make a judgement as to the cause of death and fill out Decision Form I or II. The Coordinating Center will then compare the results from both physicians. In addition, if a decision on a death is equivocal, the records will be sent to a third reviewer. The mortality review committee will arrive at a joint decision for all cases of non-concordance.

4.2.2 Data Abstraction

To assure consistency and accuracy in the chart abstractions and death certificate and medical examiner reports, a chart for morbidity, a chart for mortality, a death certificate and a medical examiner report will be circulated by the Coordinating Center to each center quarterly with personal identifiers deleted. All data abstract personnel will complete the necessary forms related to that circulated material and they will be judged at a central source for consistency and completeness.

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APPENDIX 2

Organizational Chart of the Strong Heart Study

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APPENDIX 4

Study Communities and Codes

Arizona Community Codes

County	Community Name	Community Code
Maricopa	Co-Op Colony	116
	Gila Crossing	119
	Komatke	124
	Laveen	125
	Lone Butte	127
	Komatke Hts.	799
	Maricope Colony	128
	Lehi	126
	Salt River	132
Pinal	Santa Cruz	231
	Blackwater	213
	Sacaton Flats	230
	Sacaton	229
	San Tan, Lower	232
	San Tan, Upper	233
	Stotonic	237
	Goodyear (South)	235
	Bapchule	212
	Casa Blanca	214
	Casa Blanca S	215
	Casa Blanca W	216
	Sacate	228
	Sweetwater	240

Dakotas Community Codes

County	Community Name	Community Code
Benson (Fort Totten)	Crow Hill	356
	Flora	357
	Fort Totten	358
	Knox	359
	Mission	360
	Oberon	361
	St. Michael D	362
	Tokio	363
	Warwick	364
	Woodlake Dis	365
Eddy	Hamar	396
	Sheyenne	397
Nelson	Lakota	456
	Michigan	457
	Tolna	458
Ramsey	Crary	476
	Devils Lake	477
	Doyon	478
	Lake Wood	479
Bennett (Pine Ridge)	Allen	526
	Harrington	527
	Martin	528
	Patricia	529
	Swett	530
	Tuthill	531
	Vetal	532
Dewey (Eagle Butte)	Agency Dist	605
	Bear Creek/Eagle Butte	606
	Eagle Butte	607
	Firesteel	608
	Four Bear/Swiftbird	609
	Glencross	610
	Green Grass/Eagle Butte	611
	Isabel	612
	Lantry	613
	Laplant/Swiftbird	614
	Marksville/Swiftbird	615

Dakotas Community Codes (con't.)

County	Community Name	Community Code
	Moreau River/Whitehorse	616
	Promise/Whitehorse	617
	Ridgeview	618
	Swiftbird	619
	Swiftbird JC	620
	Timber Lake	621
	Trail City	622
	White Horse	623
	Blackfoot	624
Jackson (Pine Ridge)	Belvidere	661
	Interior/Wanblee	662
	Kadoka/Wanblee	663
	Stamford	664
Jackson (Pine Ridge)	Hisle/Wanblee	846
	Longvalley	847
	Potato Creek	848
	Wanblee	849
Shannon (Pine Ridge)	Am Horse Cr/Kyle	771
	Batesland/Allen	772
	Calico	773
	Crazy Horse	774
	Cuny Table	775
	Denby	776
	Grass Creek/Manderson	777
	Kyle	778
	Lake	779
	Lakeside	780
	Manderson	781
	Oglala	782
	Pine Ridge	783
	Porcupine	784
	Red Shirt TA	785
	Rockyford/Porcupine	786
	Slim Butte	787
	White River	788
	Wolf Creek	789
	Wounded Knee/Manderson	790
	Wakfamni Lk	791

County	Community Name	Community Code
Ziebach (Eagle Butte)	Bridger/Cherry Creek	866
	Cherry Creek	867
	Dupree	868
	Glad Valley	869
	Iron Lighting	870
	Red Elm	871
	Red Scaffold	872
	Thunder Butte	873

Oklahoma Community Codes

County	Community Name	Community Code
Beckham	Carter	095
	Delhi	096
	Elk City	097
	Erick	098
	Mayfield	099
	Sayre	100
	Texola	101
Blaine	Canton	104
	Eagle City	105
	Geary	106
	Greenfield	107
	Hitchcock	108
	Homestead	109
	Longdale	110
	Okeene	111
	Southard	112
	Watonga	113
Caddo	Albert	138
	Alfalfa	139
	Anadarko	140
	Apache	141
	Binger	142
	Bridgeport	143
	Carnegie	144
	Cement	145
	Cyril	146
	Eakly	147
	Fort Cobb	148
	Gracemont	149
	Hinton	150
	Hydro	151
	Lookeba	152
	Riverside Bi	153
	Washita	154
Canadian	Concho	158
	Concho Bia S	159
	Calumet	160
	El Reno	161

County	Community Name	Community Code
	Mustang	162
	Piedmont	163
	Union	164
	Yukon	165
Carter	Ardmore	169
	Carter Semin	170
	Clemscot	171
	Fox	172
	Gene Autry	173
	Graham	174
	Healdton	175
	Lone Grove	176
	Mcman	177
	Milo	178
	Newport	179
	Pooleville	180
	Ratliff City	181
	Springer	182
	Tatums	183
	Tussy	184
	Wilson	185
	Wirt	186
	Woodford	187
Cleveland	Lexington	232
	Moore	233
	Noble	234
	Norman	235
Comanche	Cache	245
	Chattanooga	246
	Elgin	247
	Faxon	248
	Fletcher	249
	Ft. Sill BIA	250
	Geronimo	251
	Indiahoma	252
	Lawton	253
	Medicine Prk	254
	Meers	255

County	Community Name	Community Code
	Sterling	256
Cotton	Devol	260
	Randlett	261
	Temple	262
	Walters	263
Custer	Arapaho	291
	Butler	292
	Clinton	293
	Custer	294
	Moorewood	295
	Thomas	296
	Weatherford	297
Garvin	Foster	350
	Elmore City	351
	Hennepin	352
	Lindsay	353
	Maysville	354
	Paoli	355
	Pauls Valley	356
	Pernell	357
	Stratford	358
	Wynnewood	359
Grady	Alex	363
	Amber	364
	Bradley	365
	Chickasha	366
	Cox City	367
	Minco	368
	Nennekah	369
	Pocassett	370
	Rush Springs	371
	Tuttle	372
	Verden	373
Greer	Brinkman	390
	Granite	391
	Mangum	392
	Reed	393
	Willow	394
Harmon	Vinson	395

County	Community Name	Community Code
	Gould	397
	Hollis	398
Jackson	Altus	435
	Blair	436
	Duke	437
	Eldorado	438
	Elmer	439
	Headrick	440
	Martha	441
	Olustee	442
Jefferson	Addington	446
	Fleetwood	447
	Grady	448
	Hastings	449
	Oscar	450
	Ringling	451
	Ryan	452
	Terral	453
	Waurika	454
Kiowa	Cooperton	495
	Gotebo	496
	Hobart	497
	Lone Wolf	498
	Mountain Prk	499
	Mountain View	500
	Roosevelt	501
	Snyder	502
Love	Burneyville	565
	Leon	566
	Marietta	567
	Orr	568
	Overbrook	569
	Rubottom	570
	Thackerville	571
Mcclain	Blanchard	574
	Byars	575
	Dibble	576
	Newcastle	577

Oklahoma Community Codes (cont'd.)

County	Community Name	Community Code
	Purcell	578
	Rosedale	579
	Washington	580
	Wayne	581
Murray	Big Canyon	664
	Davis	665
	Dougherty	666
	Hickory	667
	Sulphur	668
Oklahoma	Okla City, Rur	717
	Arcadia	718
	Bethany	719
	Choctaw	720
	Edmond	721
	Harrah	722
	Jones	723
	Luther	724
	Newalla	725
	Nicoma Park	726
	Okla City, Urb	727
	Spencer	728
	Wheatland	729
	Del City	730
	Midwest City	731
Stephens	Alma	905
	Bray	906
	Comanche	907
	County Line	908
	Duncan	909
	Loco	910
	Marlow	911
	Velma	912
Tillman	Davidson	927
	Frederick	928
	Grandville	929

Oklahoma Community Codes (cont'd.)

County	Community Name	Community Code
	Hollister	930
	Loveland	931
	Manitou	932
	Tipton	933
Washita	Bessie	965
	Burns Flat	966
	Canute	967
	Cloud Chief	968
	Colony	969
	Cordell	970
	Corn	971
	Dill City	972
	Foss	973
	Rocky	974
	Sentinel	975

APPENDIX 5

a. Codes for IHS Facilities by Area and Service Unit

Service Area Code	Service Unit Code	Location Code	Facility Name
		Area: Aberdeen	
Service	Unit:	Non SVC Unit	
10	00	00	Aberdeen
Service	Unit:	Rapid City	
10	09	01	Rapid Ct Gms
10	09	88	R Shirt T Hs
Service	Unit:	Cheyenne Riv	
10	10	01	Eagle Butte
10	10	31	Cherry Cr Hs
10	10	33	Red Scaff Hs
10	10	34	Swiftbird Hs
10	10	35	Whitehhors Hs
10	10	51	Cherry Cr Hs
10	10	82	Faith
10	10	88	Isabel
Service	Unit:	Ft.	Berthold
10	12	00	Newtown Fo
10	12	10	Minni - Tohe
10	12	30	Mandaree Hs
10	12	31	Twin Bute Hs
10	12	32	Wh Shield Hs
Service	Unit:	Ft. Totten	
10	13	10	Ft. Totten Hc
10	13	51	St. Michaels
Service	Unit:	Pierre	
10	14	00	Chamberln Fo
10	14	21	Pierre S - Hc
10	14	30	Ft. Thomps Hs
10	14	31	Low Brule Hs
Service	Unit:	Pine Ridge	
10	15	01	Pine R'g Ho
10	15	10	Wanblee Hc
10	15	30	Allen Hs
10	15	31	Kyle Hs

Service Area Code	Unit Code	Location Code	Facility Name

Service Area Code	Unit Code	Location Code	Facility Name
10	15	32	Manderson Hs
10	15	62	Porcupine Sc
10	15	63	Porcupine Ch
Service	Unit:	Rosebud	
10	16	01	Rosebud Hosp
10	16	55	Norris Sc
10	16	59	St. Francis
10	16	61	White River
10	16	90	Parmelee
Service	Unit:	Sisset - Wahpt	
10	17	01	Sisseton Hos
10	17	22	Wahpeton Hc
Service	Unit:	Standing Rock	
10	18	01	Ft. Yates Hos
10	18	10	Mclaughln Hc
10	18	30	Bullhead Hs
10	18	31	Cannonbal Hs
10	18	33	Wakpala Hs
Service	Unit:	Turtle Mount	
10	19	01	Belcourt Hos
Service	Unit:	Omaha - Winneb	
10	20	01	Winnebago Ho
Service	Unit:	Yankton	
10	21	01	Wagner Hosp

Area: Oklahoma

Service Area Code	Unit Code	Location Code	Facility Name
Service	Unit:	Claremore	
50	52	01	Claremore Ho
50	52	14	Miami Hl Cen
Service	Unit:	Clinton	
50	53	01	Clinton Hosp
50	53	10	Watonga H. Ct
50	53	11	Concho Hc
Service	Unit:	Kansas	
50	54	10	Holton Hc
50	54	20	Haskell Hc

Service Area Code	Unit Code	Location Code	Facility Name
		Area: Oklahoma (con't.)	
50	54	30	White Cloud
Service	Unit:	Lawton	
50	55	01	Lawton Hosp
50	55	10	Anadarko Hc
50	55	30	Carnegie Hc
50	55	31	Riverside Hs
Service	Unit:	Pawnee	
50	56	10	Pawhuska Hc
50	56	11	White Eag Hc
50	56	12	Pawnee Hc
50	56	13	Pawnee Benefit Package
Service	Unit:	Shawnee	
50	58	10	Shawnee H Ct
Service	Unit:	Tahlequah	
50	59	01	W.W. Hastings
Service	Unit:	Ada	
50	62	01	Carl Albrt H
50	62	11	Wewoka Hl Ct
		Area: Oklahoma Tribe/638	
Service	Unit:	Claremore	
55	52	02	Creek Nation
55	52	11	Delaware Dhc
55	52	12	Okemah H Ct
55	52	15	Indian Hlth. Res. (Tulsa)
55	52	16	Salina Hlth. Center
55	52	31	Sapulpa Hc
Service	Unit:	Shawnee	
55	58	11	Oklahoma City Clinic
55	58	12	Black Hawk Clinic
Service	Unit:	Tahlequah	
55	59	10	Eufaula Hc
55	59	11	Sallisaw Hc
55	59	12	Dkmartin Hc
Service	Unit:	Talihina	
55	60	01	Talihina Hos
55	60	11	J. Andrsn Hc

Area Code	Service Unit Code	Location Code	Facility Name
		Area: Oklahoma (cont'd)	
55	60	12	Hugo Hl Ct
55	60	13	Mcalester Hc
Service	Unit:	Ada	
55	62	10	Tishomingo
55	62	12	Ardmore Hc
Service	Unit:	Eagle Pass	
55	63	00	Eagle Pass
		Area: Phoenix	
Service	Unit:	Keams Canyon	
60	62	01	Keams Canyon
Service	Unit:	Owyhee	
60	63	01	Owyhee Hosp
Service	Unit:	Colorado Riv	
60	64	01	Parker Hosp
Service	Unit:	Phoenix	
60	66	01	Phoenix Ho
60	66	20	Phoenix H S
60	66	30	West End H C
60	66	31	Salt River
60	66	63	Ft. McDowell Hs
60	66	99	Undesig Locs
Service	Unit:	Sacaton	
60	67	01	Asacaton Hos
Service	Unit:	San Carlos	
60	68	01	San Carlos
Service	Unit:	Schurz	
60	69	01	Schurz Hosp
Service	Unit:	Whiteriver	
60	71	01	Whiteriver H
Service	Unit:	Ft. Yuma	
60	72	01	Ft. Yuma

b. Non-IHS Hospitals and Codes

1. Dakota

Pine Ridge

Martin Hospital	10-15-55
Kadoka Hospital	10-15-56
Philip Hospital	10-15-57
Hot Spring VA Hospital	10-15-58
Ft. Meade VA Hospital	10-15-59
Rapid City Regional Hospital	10-15-60
Gordon, Nebraska Hospital	10-15-61
Porcupine Community Clinic	10-15-62
University of Minnesota Hospital	10-15-63
Hot Spring Community Hospital	10-15-64
Fitzsimons Hospital, Denver	10-15-65
Sioux Valley Hospital, Sioux Falls	10-15-66
McKennan Hospital	10-15-67
Ellsworth AFB	10-15-68
Wall Clinic	10-15-69
Rapid City Eye Institute	10-15-70
Minneapolis VA Medical Center	10-15-71
St. Anthony Hospital, Denver	10-15-72
Porter Memorial Hospital	10-15-73

Eagle Butte

Faith Clinic	10-10-82
Isabel Clinic	10-10-83
St. Mary's Hospital, Pierre	10-10-84
Sacred Heart, Yankton	10-10-85
Mid Dakota, Chamberlain	10-10-86
Med Center One, Bismarck, ND	10-10-87
St. Alexius, Bismarck, ND	10-10-88
Mobridge Hospital	10-10-89
Gettysburg Hospital	10-10-90

Ft. Totten

Mercy Hosital, Devil's Lake	10-10-60
New Rockford Hospital	10-10-61
United Hospital, Grand Forks	10-10-62
St. Lukes Hospital, Fargo	10-10-63
Fargo VA Hospital	10-10-64

2. Phoenix

Desert Samaritan Hospital	60-66-70
Good Samaritan Hospital	60-66-71
Humana Hospital	60-66-72
Jesse Owens Med. Ctr.	60-66-73
John C. Lincoln Hospital	60-66-74
Maricopa Med. Ctr.	60-66-75
Maryvale Samaritan Hospital	60-66-76
Mesa Lutheran Hospital	60-66-77
Phoenix Baptist Hospital	60-66-78
Phoenix Memorial Hospital	60-66-79
St. Luke's Medical Center	60-66-80
Scottsdale Memorial Hospital	60-66-81
St. Joseph's Hospital	60-66-82
Valley Lutheran Hospital	60-66-83
Chandler Community Hospital	60-66-84
NIH	60-66-85
Family Care Clinic in Chandler	60-66-86

3. Oklahoma

Anadarko Municipal Hospital	08-01-14
Carnegie Tri-County Municipal Hospital	08-02-14
Comanche County Memorial Hospital	16-01-05
Southwestern Medical Center	16-03-05
Reynolds Army Hospital	16-05-05
Grady Memorial Hospital	26-01-14
Veterans Administration Hospital	55-18-01
State of Oklahoma Teaching Hospitals	55-20-01
Oklahoma Memorial Hospital	55-20-01
Oklahoma Childrens Memorial	55-20-01
Duncan Regional Hospital	69-28-76
Mercy Hospital	55-63-76
South Community	55-63-87
Norman Regional Hospital	14-60-24
St. Anthony Hospital	55-63-78
Baptist Medical Center	55-63-89
Deaconess Hospital	55-63-24
Presbyterian Hospital	55-63-84
Midwest City Memorial Hospital	55-53-29

APPENDIX 6 (a)

PERSONNEL CODES

ARIZONA CENTER

301	Barbara Howard
303	Jim Howard
304	Betty Jarvis
305	Paula Harper
307	Linda Phillips
309	Michael Paidi
310	Shinkuro Kataoka
312	Joy Jones
313	Matilda Johns
314	Helen Johns
315	Rowena Juan
316	Oreen Johns
317	Angeline Barley
320	Sonja Antone
321	Carmelita Estenson
322	Maurice Sievers
323	Rosinna Brionnes
327	Daryl Allis
328	Greg Wood
329	Don Warne
333	Andrea Kriska
339	Kathleen Quintero
340	Robert Hanson
341	Janet Andrews
342	Joanne Carter
343	Michael Cyll
344	Veronica Rhodes
345	Valentine Rodriguez
346	Wesley Yamada
347	Judy Bergman
348	Pamala Daily

DAKOTAS CENTER

101	Thomas K. Welty
102	Beverly Blake
103	Monique Brave
104	Dianne Rand
105	Cecilia Fire Thunder
106	Eileen Iron Cloud
107	Pauletta Red Willow
108	Sharon Eagle
109	Arliss Keckler
110	Jackie Hallevoet
111	Mary Clark-Lee
112	Shirley Butts
113	Veronica Williams
114	Vincent Whipple
115	Linda Comini
116	Gregory Cost
117	Christine Nichols
118	Georgia McIntosh
119	Corbin LeBeaux
120	Pauline Livermont
121	Lakota Kruse
122	Gregory Shuford
123	Gayle Moran
124	Geoffrey Fitzgerald
125	Arvo Oopik
126	Mary Clown
127	Geri Shangreaux
128	Joanne Berry
129	Victoria Standing Elk
130	Lydia Alvarez
131	Amy Livermont
132	Debra Bruguier
133	Betty Cook
134	Kurt Schweigman
135	Rose White Face
136	Billy Beets

137	Hanky Poafpybitty	180	Claudia Keidl
138	Linda Otero	181	Neva Zephier
139	Evelyn Chaske	182	Lillian Brown
140	Juanita Young	183	Craig Baker
141	Betty Johnson	184	Mike Karch
142	Linda Gibbons	185	LaMena Monteiro
143	Annabelle Weston	186	Juanita Mauer
144	Claudia Roeber	187	Shelly Moran
145	Jeri Archer	188	Helen Beaty
146	Jennifer Gireaux	189	Jenny Lawrence
147	Mary Lou Jetty	190	Alan Crawford
148	Norman Blue Arm	191	Barbara Paulson
149	Pearl Rodriguez	192	Mark E. Dorogy
150	Charles White		
151	Kim Smith		
152	Patti Lesmeister		
153	Bonita Claymore		**OKLAHOMA CENTER**
154	Will Knight		
155	Amy Koplovsky	201	Elisa Lee
156	Kristone Griffith	202	Linda Cowan
157	Kevin McNeill	204	Jeunliang Yeh
158	Jane Runzheimer	205	Oscar T. Go
159	Geneva Lone Hill	208	Linda Poolaw
160	Marcia O'Leary	213	Yvonne Kobus
161	Susan Baker	214	Lois Swift
162	Jamie Giroux	215	Juanita Cortez
163	Andrew Swietlik	216	Sherry Jackson
164	Andre Winter	220	Martha Stoddart
165	Alicia Rosen	222	Tae Young Heo
166	Kathlenn Jenkins	227	Tauqeer Ali
167	Robert Stern	228	Brenda Matsler
168	Gillian Schweitzer	229	Karen Kimbley
169	Deanne Miner	231	Stephanie Gomez
170	Marge Grant	232	Jie Chen
171	Lawrence Sokoloff	233	LeeAnn Bruised Head
172	Jackie Sokoloff	234	Dora Resendez
173	Verlean Ice	235	Leon Kalbfleisch
174	Mona Brave	250	Richard Devereux
175	Sue Rooks	251	Cheryl Pegus
176	Daniel Jetty	270	Paul Enright
177	Patricia Foote		
178	Janice Idland		
179	Valarie Brown		

Handwritten notes:
Dr Saul
Sudah Nair
Sabeeh Jaffery −239
Fawn Yeh
Wenyu Wang
Joss Langevin
Bill Moore
Carl Schaefer
Kavita
Boram Sambo −242
Denirsh −261
Camain Rafiq −262
Yn-Jiun

APPENDIX 6 (b)

THE STRONG HEART STUDY II
Cardiovascular Disease in American Indians

Confidentiality Pledge

I, _____ understand that data obtained for subjects of research projects are confidential.

I will not reveal to unauthorized persons any patient's name or any identifying information or any other information obtained from subjects of the project entitled, "Cardiovascular Disease in American Indians (The Strong Heart Study)".

I will not allow any persons who are not authorized members of the Strong Heart Study staff to have access to any information collected from or about the subjects.

I will properly store the data forms, computer printouts and other documents in locked file cabinets or drawers to protect confidentiality.

I understand that breach of this confidentiality pledge is grounds for dismissal from employment on the Strong Heart Study.

I will return all data to the Principal Investigator when my employment terminates.

_____
Staff Member

_____
Principal Investigator

_____
Date

THE STRONG HEART STUDY II
CARDIOVASCULAR DISEASE IN AMERICAN INDIANS

Mortality Survey
Death Certificate Form

ID number:

Community Code:

Social Security Number:

Information from death certificate:

1. Decedent:

    a. Last name:

    b. Middle name:

    c. First name:

2. Death certificate number: (*State File Number*)

3. Sex: (1=Male/ 2=Female)

4. Race/Ethnicity:
   (1=American Indian/ 2=Hispanic/ 3=White/ 4=Black/ 5=Oriental/ 8=Other/ 9=Unknown)

5. Marital status:
   1= married
   2= single
   3= separated
   4= divorced
   5= widowed
   9= unknown

6. Date of birth:
   mo   day   yr

7. Date of death:
   mo   day   yr

8. Age at death:

9. Time of death (*24 hour clock*):
   (*If "Death Occurred" is missing use "Death Pronounced"*)
   hr : min

10. Where did the decedent die?   ☐
     1= IHS hospital/clinic in study area
     2= non-IHS hospital in study area
     3= hospital out of area
     4= home
     5= other, _____
     9= location unknown

If died in hospital, fill out Question 11, otherwise skip to Question 12.

11. Name of hospital/clinic or nursing home where death occurred or pronounced.

     a. Name: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

     b. City/Reservation: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

     c. State-Zip: ☐☐ ☐☐☐☐☐

12. Was an autopsy performed ?   (1=yes, 2=no, 9=unknown)   ☐

13. Was this a coroner's or medical examiner's case?   (1=yes, 2=no, 9=unknown)   ☐

14. If yes, coroner or medical examiner:

     a. Last name: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

     b. First name and middle initial: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

     c. Street address: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

     d. City: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

     e. State-Zip: ☐☐ ☐☐☐☐☐

15. Interval between onset and death (*for immediate cause of death*):   ☐

     1= 5 min. or less
     2= 1 hour or less
     3= 1 day or less
     4= 1 week or less
     5= 1 month or less
     6= more than 1 month
     9= unknown or not recorded

16. Informant listed on death certificate:

    a. Last name: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

    b. First name and middle initial: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

    c. Street address: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

    d. City: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

    e. State-Zip: ☐☐ ☐☐☐☐☐

17. Relationship of informant to decedent: ☐
    1= spouse
    2= other relative, specify: _____
    3= non-relative, friend
    9= unknown

18. Certifying physician:

    a. Last name: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

    b. First name and middle initial: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

    c. Street address: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

    d. City: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

    e. State-Zip: ☐☐ ☐☐☐☐☐

19. Date abstract completed: ☐☐ / ☐☐ / ☐☐
                                   mo   day   yr

20. Code number of abstractor completing this form: ☐☐☐

INSTRUCTIONS OF DEATH CERTIFICATE FORM

I.   GENERAL INSTRUCTIONS

The Death Certificate Form is completed for each eligible death. The purpose of this form is to obtain information on the decedent and information on the informant, coroner/medical examiner, or certifying physician. The ID number has 6 digits: the first digit identifies center (l=SD, 2=OK, 3=AZ), the second digit identifies vital status (l=dead, 0=alive), and the last four digits identifies the individual. For this form, the second digit is 1 for all subject. The community code has 3 digits, it is the standard IHS community code.

II.   DETAILED INSTRUCTIONS FOR EACH QUESTION

Item	Instructions

1.   Decedent's name. Enter the first, middle, and last name of the decedent. Begin each name in the left-most box using CAPITAL letters.

2.   Death certificate number. This number will be found stamped or typed on the death certificate. If a computer printout is used, it must include this information. Record the number starting in the right-most box. DO NOT add zero to the right of the number.

3.   Sex. Record the decedent's sex.

4.   Race. Record as is stated.

5.   Marital status. Record as listed. If the death certificate just says "not married" or "S", record as "Single".

6.   Date of birth. Record as listed on the death certificate.

7.   Date of death. Record as listed on the death certificate.

8.   Age at death. If the age at death is recorded on the death certificate, check it by using the following algorithm. Also if age is not recorded, then use this algorithm to compute it.

   a.   If the month and day of birth fall BEFORE the month and day of death, subtract the year of birth from the year of death.

   b.   If the month and day of birth fall AFTER the month and day of death, subtract (year of birth + 1) from the year of death.

   Record the correct age on the form. If the age at death cannot be computed then enter "=" in each field.

9. Time of death. Convert all time to 24 hour clock and record. Enter unknown as "=" in each field.

10. Location of death. Choose an appropriate answer. Other includes nursing home, another residence, or a non-hospital institution.

11. Name of hospital/clinic/nursing home/non-hospital institution/another residence where death occurred. Enter the name and location of the location where the decedent died. Include the city and state.

12. Coroner/medical examiner's case. Record as indicated on the death certificate.

13. Name and location of coroner/medical examiner. Record the name and address of the coroner/medical examiner who signed the death certificate. Provide as much detail as is recorded on the death certificate.

14. Autopsy. Record as indicated on the death certificate.

15. Interval of onset of symptoms and death. Record the shortest possible category for the immediate cause of death as indicated on the death certificate. If this is missing, DO NOT substitute the interval for another cause. Instantaneous should be recorded as "5 minutes or less".

16. Informant. Most death certificates have a line for the informant. Often this is the spouse, but it may be a co-worker, etc. Record the name and address. Provide as much detail as is recorded on the death certificate.

17. Relationship of informant. Recorded as listed on the death certificate. If no information is provided then record as "unknown".

18. Certifying physician. Record the name and address of the certifying physician who signed the death certificate, if not the coroner or medical examiner.

19. Date abstract completed. Record the date the Death Certificate Form is completed.

20. Code number of abstractor. The field center staff member who has completed this form must enter his/her valid Strong Heart Study code number in this question.

APPENDIX 8
STRONG HEART STUDY II
CARDIOVASCULAR DISEASE IN AMERICAN INDIANS

PHOTOCOPY CHECKLIST FOR MEDICAL RECORDS REVIEW
FATAL CVD EVENT (Page 1)

Admission date: _____   ID Number: [   ]

For each hospital admission WITHIN the YEAR prior to death, obtaining photocopies of each of the following sections of the medical history (when available) and <u>assemble them for each admission.</u> Be sure that photocopies are legible.

	YES	NO	DONE, but Report Not Available
Admission Sheets (Face Sheets)	___	___	___
Admitting History and Physical Exam	___	___	___
Discharge Summary	___	___	___
ECGs (within past 2 years)	___	___	___
Reports of results of:			
Chest X-ray	___	___	___
Echocardiogram	___	___	___
Angiogram	___	___	___
Exercise tolerance test (Treadmill)	___	___	___
Cardiac catheterization	___	___	___
CT (CAT) scan - head	___	___	___
MRI - head	___	___	___
Carotid ultrasound	___	___	___
Lumbar puncture	___	___	___

PHOTOCOPY CHECKLIST FOR MEDICAL RECORDS REVIEW FATAL CVD EVENT (Page 2)

ID Number: [   ]

	YES	NO	DONE, but Report Not Available
Operative reports:			
Coronary bypass	____	____	____
Angioplasty	____	____	____
Swan-Ganz catheterization	____	____	____
For terminal Event Only:			
Ambulance report	____	____	____
ER Admission and Discharge Summary	____	____	____
Any clinical notes regarding DOA	____	____	____
Autopsy Report/ Coroner's Report	____	____	____

Abstractor Number [ ][ ][ ]

Date abstract completed: [ ][ ]/[ ][ ]/[ ][ ]
                                   mo   day   yr

APPENDIX 9
THE STRONG HEART STUDY II
CARDIOVASCULAR DISEASE IN AMERICAN INDIANS

Mortality Survey -- Medical Records Abstract

Medical charts (IHS and/or other community hospitals) of all potential CVD decedents will be reviewed. Complete this form if the decedent had the following ICD-9 codes listed as the underlying cause of death or contributing cause of death and had been hospitalized within 1 year prior to the death : 250, 390-448, 518.4, 585, 798, 799. Use all available medical records to complete this form. All photocopies must be legible.

ID Number:

Community code (*see instruction*):

Social Security Number:

1. Degree of Indian Blood, if know the fraction, record it.

    If not, record the code appear on the face sheet of the chart (*Item 7-Optional*)

    What is decedent's tribe of enrollment ? (*Using IHS tribal code*)

2. Has the decedent been hospitalized within the year prior to his/her death ?
    1=yes,　　　2=no.

3. What was the date of the latest outpatient or ER visit?
    (*Do not include Death on Arrival (DOA).*)
    　　　　　　　　　　　　　　　　　　　mo　　day　　yr

4. Is there a history of a prior myocardial infarction?
    (*Not including the event precipitating the death*).
    1=yes,　　　2=no,　　　8=uncertain,　　　9=not mentioned

5. If yes, date of most recent event :
    　　　　　　　　　　　　　　　　　　　mo　　day　　yr

    Facility where hospitalized: _____

6. Is there a history of stroke ?
    1=yes,　　　2=no,　　　8=uncertain,　　　9=not mentioned

7. if yes, date of most recent event:
    　　　　　　　　　　　　　　　　　　　mo　　day　　yr

    Facility where hospitalized: _____

8. Is there a history of congestive heart failure ?
    1=yes,   2=no,   8=uncertain,   9=not mentioned   ☐

9. If yes, date of most recent event: ☐☐/☐☐/☐☐
                                                  mo   day   yr

    Facility where hospitalized: _____

10. Were any 12 lead ECG's taken during the two years prior to death?   ☐
    1=yes   2=no
    If ECGs were taken, attach copies of required ECGs and interpretations.

11. List all facilities where the patient was hospitalized since January 1, 1989.
    If decedent was a Phase I participant, complete Morbidity Medical Review Form.

    Reason: 1=Heart attack 2=Stroke 3=CHF 4=Other, please specify. _____

	Hospital/Clinic	Town/State	Date (mo/day/yr)	Reason
i.	_____	_____	___/___/___	☐
ii.	_____	_____	___/___/___	☐
iii.	_____	_____	___/___/___	☐
iv.	_____	_____	___/___/___	☐
v.	_____	_____	___/___/___	☐
vi.	_____	_____	___/___/___	☐
vii.	_____	_____	___/___/___	☐
viii.	_____	_____	___/___/___	☐
ix.	_____	_____	___/___/___	☐
x.	_____	_____	___/___/___	☐

12. Usual IHS facility: _____   ☐☐☐☐☐

    IHS Chart Number: ☐☐☐☐☐

13. Hospitalization within 1 year of death: 1=Yes 2=None   ☐

        Enter the ICD-9 code numbers for the hospital discharge diagnoses and procedure
    codes recorded in all medical records of hospitalization within one year of death, exactly as
    they appear on the front sheet of the medical record and/or on the discharge summaries. Be
    sure they are ICD-9 codes. Record diagnoses if no codes are available.

1. ☐☐☐·☐☐   6. ☐☐☐·☐☐

2. ☐☐☐·☐☐   7. ☐☐☐·☐☐

3. ☐☐☐·☐☐   8. ☐☐☐·☐☐

4. ☐☐☐·☐☐   9. ☐☐☐·☐☐

5. ☐☐☐☐·☐☐   10. ☐☐☐·☐☐

14. During the terminal hospitalization, were any cardiac enzyme tests done within DAYS ☐
1-4 after arrival at the hospital or after in-hospital coronary heart disease event ?
(1=yes, 2=no, 8=uncertain, 9=not mentioned)

RECORD THE ENZYME TEST RESULTS ON THE FOLLOWING PAGE

15. Abstractor Number   ☐☐☐

16. Date abstract completed: ☐☐/☐☐/☐☐
                                      mo   day   yr

CARDIAC ENZYME TEST RECORDS

Hospital Admission Date: _____ / _____ / _____   Complete Separate Forms for Each Admission in Which Enzyme Were Done.

SPECIFY THE UNIT OF MEASUREMENT	DAY ONE (DATE:)			DAY TWO (DATE:)			DAY THREE (DATE:)			DAY FOUR (DATE:)		
	No.1	No.2	No.3	No.1	No.2	No.3	No.1	No.2	No.3	No.1	No.2	No.3
Total CK (CPK)												
Upper Limit of Total CK (CPK)												
CK-MB												
Upper Limit of CK-MB												
Total LDH												
Upper Limit of Normal LDH												
LDH1												
LDH2												
LDH1/LDh2												
SGOT												
Upper Limit of Normal SGOT												

INSTRUCTIONS FOR MORTALITY SURVEY:
MEDICAL RECORDS ABSTRACT

I.   GENERAL INSTRUCTIONS

The Medical Records Abstract for the Mortality Survey is completed, either partially or in toto, for each death with a mention of any type of cardiovascular disease on the death certificate. The purpose of the abstract and photocopying is to provide sufficient information to allow the underlying cause of death to be classified by the Event Committee. Because some deaths will occur outside the hospital, Questions 4-7 on the abstract form are intended to collect information on the decedent's medical history when there was no hospitalization within 1 year prior to death. The remainder of the form collects information from hospitalizations that occurred within 1 year of death, including the terminal hospitalization. Review all available medical records to complete this form.

If the decedent participated in Phase I, their SHS Phase I ID number should be used. Otherwise, an ID number will be assigned according to the same procedures used in Phase I: the first digit identifies the study center (1=SD, 2=OK, 3=AZ), the second digit identifies vital status (1=dead, 0=alive), and the last four digits identify the individual. For this form, the second digit is 1 for all subjects. The community code has 3 digits and is the standard IHS community code.

II.   DETAILED INSTRUCTIONS FOR EACH QUESTION

Item   Instructions

Enter the study ID number, community code, patient's social security number and degree of Indian blood recorded in chart. If the patient did not have a social security number, draw double lines through the boxes.

1.   Find out decedent's Indian blood from face sheet of the IHS Hospital/Clinic chart.

2.   If the decedent was hospitalized within one year of death, including the terminal hospitalization, this should be coded as 1 (yes). If the hospitalization you are abstracting occurred more than one year prior to death, code as 2 (no).

3.   Record the date (month, day, last two digits of year) of the last outpatient or emergency room visit prior to death. Choose the one closest to the date of death, i.e., the most recent, but do not count the ER admission at which a person was DOA.

4.   This item is intended to identify those decedents who had a history of myocardial infarction prior to the terminal or last admission. If the admission you are abstracting was for a MI, do not count it when answering this question. In this and other questions, "yes" means that a positive history or occurrence is specifically mentioned in the record, "no" means that the absence of the event or occurrence is specifically stated in the record (e.g. "there is no previous history of heart disease in this patient"), "uncertain" means that the medical record includes some qualifying adjectives or statements, such as "possible" or "there is some suggestive of a prior history, but this is unclear" and so on, "not mentioned" means that nothing regarding the event or occurrence is mentioned in the medical record.

5.	If the decedent had a definite or uncertain history of prior MI (codes 1 and 8 in Question 4), enter the date of the most recent MI and print the name of the facility where he/she was hospitalized. If the event occurred between 1989-1994 and is listed as: acute MI or R/O MI (ICD code 410) or acute coronary insufficiency (ICD code 411), complete a NEW MI Form if the decedent was a Phase I participant.

6.-12.	Questions 6-9 ask about the decedent's history of selected diseases and medical procedures. This information may be given in the admitting history and physical examination of the index admission, but it may also be necessary to check these sections and the discharge sheet for all prior admissions included in the record. After review of the record for each of these items, record the appropriate response, again using the definitions of yes, no, uncertain and not mentioned given above.

Question 10 concerns 12-lead ECGs, code as instructed. If yes, attach copies of required ECGs and interpretations. These include the last ECG obtained prior to the last admission, the first ECG done after admission, the first ECG done each day thereafter, and the last ECG obtained prior to death or discharge. Be sure that personal identifiers are removed, but also that the ID number is recorded on each ECG attached. Be sure that ECGs are dated so that it is possible to determine the order in which they were done.

The requested photocopies on the check list should be made and attached to the abstract.

Enter the primary IHS number of the decedent. If there was an ECG done within the past 2 years and filed in the chart, attach a photocopy of the tracing and interpretation to the completed abstract. Be sure that the tracing is dated and that personal identifiers have been removed during the photocopying process. Be sure that the ID number is printed on the photocopy.

IF THERE ARE NO HOSPITALIZATIONS FOR THE DECEDENT WITHIN ONE YEAR OF DEATH, THIS FORM IS FINISHED, AND YOU MAY STOP ABSTRACTING.

13.	Print the ICD-9 codes of the discharge diagnoses and procedures as listed on the front sheets of the admissions you are abstracting. Record them in the order they are listed in the record. Be sure these are ICD codes and not another coding system. If the codes are not ICD-9, leave the boxes blank and print the diagnosis next to each box. The ICD-9 codes will be done at the Coordinating Center. If the patient died in the hospital and an autopsy report is included in the chart, code as 1 (yes) and attach a photocopy of the autopsy report. Again, be sure to omit personal identifiers from the photocopy, but print the ID number on the copy.

14.	Determine whether any cardiac enzyme studies were done within 1-4 days after arrival at the hospital or after an in-hospital cardiac event for the terminal admission. Cardiac enzymes include CPK (CK), LDH, and SGOT.

If cardiac enzymes were done during the appropriate time frame after the event, complete the Enzyme Test Form(s).

Complete the photocopy check list and assemble materials. Be sure to include the units of measurement for each enzyme. If these are not given, contact the laboratory where the tests were done and ask for the information. Again, you may have to contact the lab to obtain this information. Since laboratories change their normal limits over time, be sure they correspond to the dates the tests were done.

In some cases, information about cardiac enzymes may only be available from discharge summaries, i.e., no lab slips are available. In this circumstance, record the information available from the discharge summary on the enzyme form, and indicate at the top of the enzyme form that these data come from the discharge summary only.

16.   Enter your code number.

14.   Enter the date the abstract was completed.

APPENDIX 10
THE STRONG HEART STUDY II

Mortality Survey -- Physician Questionnaire

ID number:

Community Code:

Social Security Number:

1. Decedent's information (filled by each study center):

a. Name: _____
         Last   *First*   *Middle*

b. Decedent's address: _____

_____

c. Date of death: _____

A. If you have any of the following information, please send us these photocopies:

	YES	NO	DONE, but Report Not Available
Admission Sheets (Face Sheets)	___	___	___
Admitting History and Physical Exam	___	___	___
Discharge Summary	___	___	___
ECGs (within past 2 years)	___	___	___
Reports of results of:			
Chest X-ray	___	___	___
Echocardiogram	___	___	___
Angiogram	___	___	___
Exercise tolerance test (Treadmill)	___	___	___
Cardiac catheterization	___	___	___

	YES	NO	DONE, but Report Not Available
CT (CAT) scan - head	___	___	___
MRI - head	___	___	___
Carotid ultrasound	___	___	___
Lumbar puncture	___	___	___

Operative reports:

	YES	NO	DONE, but Report Not Available
Coronary bypass	___	___	___
Angioplasty	___	___	___
Swan-Ganz catheterization	___	___	___

For terminal Event Only:

	YES	NO	DONE, but Report Not Available
Ambulance report	___	___	___
ER Admission and Discharge Summary	___	___	___
Any clinical notes regarding DOA	___	___	___
Autopsy Report/ Coroner's Report	___	___	___

APPENDIX 11

THE STRONG HEART STUDY II
CARDIOVASCULAR DISEASE IN AMERICAN INDIANS

Mortality Survey
Informant Interview Form

ID number:

Community Code:

Social Security Number:

A. DECEDENT (*filled by study center staff*)

1. Name: _____
    _____Last_____First_____Middle_____

2. Date of death: _____

B. INFORMANT--*from death certificate, filled by study center staff.*

3. a. Name: _____
    _____Last_____First_____Middle_____

    b. Address: _____

    _____

    c. Telephone: (_____) _____ — _____

C. RECORD OF CALLS or HOME VISIT

	DATE (mo/day/yr)	TIME (24 hr clock)	Method of contact 1=Phone 2=Home Visit 3=Other	Contact successful 1=Yes 2=No	Interview Completed 1=Yes 2=No
1)	__/__/__	_____	☐	☐	☐
2)	__/__/__	_____	☐	☐	☐
3)	__/__/__	_____	☐	☐	☐
4)	__/__/__	_____	☐	☐	☐
5)	__/__/__	_____	☐	☐	☐

4. Before we get started, could you please tell me what was your relationship to the deceased?

<div style="text-align:right">☐</div>

       1=spouse,
       2=parent,
       3=daughter/son,
       4=other relative,
       5=friend,
       6=work-mate,
       other, _____

5. Do you know the degree of Indian blood of _____ ?
Write down the fraction of Indian blood. If unknown record 99/99.

<div style="text-align:right">☐☐ / ☐☐</div>

6. First, think back to about one month before (_____) died. At that time, was he/she sick or ill, with his/her activities limited, or was he/she normally active for the most part?
1=sick/ill/limited activities,   2=normally active,   3=unknown.

<div style="text-align:right">☐</div>

7. Was (_____) being cared for at a nursing home, or at another place at the time of death ?

<div style="text-align:right">☐</div>

    1= yes, nursing home,
    2= yes, at home,
    3= yes, other, specify: _____
    4= no,
    9= unknown

8. *(If the decedent was cared by nursing home prior to the death)*, please tell me the name and location of the nursing home:

      a. Name: _____

      b. Address: _____

        City/town: _____

        State-Zip: _____

9. Was (_____) hospitalized during the year prior to death?
1=yes, 2=no, go to Question 14,   9= unknown, go to Question 14.

<div style="text-align:right">☐</div>

if yes, how many times was she/he hospitalized?

<div style="text-align:right">☐☐</div>

10. Were any hospitalizations for heart attack or chest pain?
(1=yes, 2=no, 9=unknown)

<div style="text-align:right">☐</div>

11. Was a hospitalization for heart surgery?   (1=yes, 2=no, 9=unknown)

<div style="text-align:right">☐</div>

12. What was the date of the last hospital admission?

<div style="text-align:right">☐☐ / ☐☐ / ☐☐
mo   day   yr</div>

13. Could you tell me the name and location of the hospital?

   a. Name: _____

   b. Address: _____

      City/town: _____

      State-Zip: _____

14. Was (_____) seen by a physician anytime in the year prior to death? ☐
    (1=yes, 2=no, 9=unknown)

15. Could you tell me the name and address of this physician?

   a. Last Name: _____

   b. First Name: _____

   c. Street address: _____

      City/town: _____

      State-Zip: _____

16. Could you tell me the name and address of (_____)'s
    usual physician. (If same as Q15 record as "same").

   a. Last Name: _____

   b. First Name: _____

   c. Street address: _____

      City/town: _____

      State-Zip: _____

17. Before (_____)'s final illness, had he/she ever ☐
    had pains in the chest from heart disease, for example, angina pectoris?
    (1=yes, 2=no, 9=unknown) If no, go to Question 19.

18. Did (_____) ever take nitroglycerin for this pain? ☐
    (1=yes, 2=no, 9=unknown)

19. Did a doctor ever say that (_____) had a heart attack ☐
    prior to his/her final illness?   (1=yes, 2=no, 9=unknown)

20. Was (_____) hospitalized for a heart attack? ☐
    (1=yes,   2=no,   9=unknown)

21. Did (_____) ever have a coronary bypass operation, balloon angioplasty, or some other operation or procedure to improve circulation of blood to the heart? (1=yes, 2=no, 9=unknown) □

22. For the following questions, (1=yes, 2=no, 9=unknown).

   a. Did (_____) ever have heart failure? □

   b. Did (_____) ever have rheumatic heart disease? □

   c. Did (_____) ever have any other heart disease or heart condition before his/her final illness? □

   If yes, what was the heart disease or heart condition? _____

23. Did (_____) ever have a stroke? □
    1= yes,   2= no, if no, go to Question 24

24. Did he/she have a stroke within four weeks of his/her final illness? (1=yes, 2=no) □

25. Were you present when (_____) died? (1=yes, 2=no) □

26. If no, how long before he/she died did you last see him/her? □
    1=1 hour or less,   2=24 hours or less,
    3=more than 24 hours,   9=unknown

    Did anyone see or hear (_____) when he/she died? □
    (1=yes,   2=no,   9=unknown)
    If yes, Name & address of this person.
27. How long after (_____) was last known to be alive □
    was he/she found dead? (*Enter the shortest interval known to be true*)
    1=5 minutes or less,   2=1 hour or less,   3= 24 hours or less,
    4=more than 24 hours,   9= unknown

28. Did (_____) experience pain or discomfort in his/her □
    chest, left arm or shoulder or jaw either just before death or within 3 days
    (72 hours) of death? 1=yes,   2=no,   9=unknown
    If answer is "NO" or "Unknown", skip to Question 32.

    The next set of questions deal specifically with the last episode (that led to the death) of
    (_____)'s pain or discomfort. The last episode is defined as
    starting at the time (_____) noticed discomfort that caused him/her to stop or
    change what he/she was doing.

29. Did (_____)'s last episode of pain or discomfort □
    specifically involve the chest? (1=yes,   2=no,   9=unknown)

30. Did he/she take nitroglycerine because of this last episode of pain or discomfort? □
    (1=yes,   2=no,   9=unknown)

31. How long was it from the beginning of (_____)'s
last episode of pain or discomfort to the time he/she stopped breathing on
his/her own? (*use the shortest interval known to be true*)
1= 5 minutes or less,   2= 10 minutes or less,
3= 1 hour or less,   4= 24 hours or less,
5= more than 24 hours,   9= unknown

☐

32. Within 3 days of death, or just before (_____)
died, did any of the following symptoms begin for the first time:
(1=yes,   2=no,   9=unknown)
a. Shortness of breath?

☐

b. Dizziness?

☐

c. Palpitations (pounding in the chest)?

☐

d. Marked or increased fatigue, tiredness, or weakness?

☐

e. Headache?

☐

f. Sweating?

☐

g. Paralysis?

☐

h. Loss of speech?

☐

i. Attack of indigestion or nausea or vomiting?

☐

j. Other? specify: _____

☐

The next few questions are concerned with emergency medical care (_____) may
have received prior to or at the time of death. You may have already given this information in an
answer to an earlier question. Since it is important to obtain information specifically on emergency
medical care, I hope you don't mind if these questions seem repetitive.

33. Was (_____) taken to a hospital?   (1=yes, 2=no)

☐

34. If yes, could you tell me the name and location of this hospital:

a. Name: _____

b. Street address: _____

City/town: _____

State-Zip: _____

35. Is there someone else whom we could contact, who might know more
about the circumstances surrounding (_____)'s death
or his/her usual state of health ? (1=yes,   2=no,   9=unknown)

☐

36. If yes, could you tell me the name, address, and telephone number of this person?

    a. Last Name: _____

    b. First Name: _____

    c. Street address: _____

    City/town: _____

    State-Zip: _____

    Telephone: _____

37. How was he/she related to the deceased ?
    1= spouse,   2= parent,
    3= daughter/son,   4= other relative,
    5= friend,   6= work-mate,
    7= other, _____ □

38. Finally, I want to ask you if you can tell me anything else about the
circumstances surrounding (_____)'s death,
specifically, could you please tell me what you know of
(_____)'s general health, health on the day he/she
died, and of the death itself? (1=yes,   2=no,   9=unknown) □

39. If yes, specify: General health, health on day of death, and circumstances of death:

_____

_____

_____

_____

_____

_____

_____

Interview is over, go to Question 40. To be completed immediately after the interview.

40. Did informant agreed to provide consent to gather further information?
    1=yes,   2=no,   3=not applicable □

If yes, ask the informant to sign the consent form for us to review the decedent's medical records.

41. Did the respondent frequently contradict himself/herself or give information that he/she would have no way of knowing?   (1=yes,   2=no)   ☐

42. Did the respondent seem to be reluctant to answer questions and thus might not have given all the information the interviewer would wish to know? (1=yes,   2=no)   ☐

43. On the basis of these questions, give your rating of the reliability of the interview: 1=good,   2=fair,   3=poor   ☐

44. Would you like to add other details concerning the quality of the interview? (1=yes,   2=no)   ☐

    If yes, specify: _____

45. Interviewer number: ☐☐

46. Date of data collection (month/day/year):   ☐☐/☐☐/☐☐
                                                   mo   day   yr

INFORMANT INTERVIEW FORM INSTRUCTIONS

I.   GENERAL INSTRUCTIONS

The purpose of the informant interview is to obtain information about possible cardiovascular events in order to classify the cause of death. The interview with next-of-kin is potentially difficult because of the sensitive nature of a relative's death and the difficulty recalling or understanding the events related to the death. Even if the informant initially claims no knowledge, begin the form to see if the questions can be answered.

The interviewer should enter the information required on the first page before the contact is made with the informant, though some of the informant data may need to be filled after contact, such as relationship to the decedent. In some cases the informant may change after calling, as in the case where a spouse is to be contacted but the actual informant is a son or daughter. A record of calls should be maintained for the attempts at contacting the informant. The interviewer should put the date and time of each call, any explanatory notes, a result code for each call, and the interviewer's assigned code number. Eight attempts to contact an informant should be made over a two-week period. If no contact is made, repeat in a month.

The questionnaire is divided into sections. The first is concerned with the decedent's medical history, including previous hospitalizations. Then the questions address the events immediately surrounding the fatal event, and Section C is concerned with the symptoms the deceased experienced prior to the event. Then emergency medical care is ascertained, and the information about other potential informants is requested. Finally the interviewer is asked questions about the reliability of the information obtained during the interview.

Almost all questions have multiple choices for answers; however, if necessary the interviewer can write any additional information or comments that may be important to understanding the response in the margins next to the question. A few questions require the interviewer to write out descriptions of the death or the decedent's state of health as related by the informant. For these questions, the interviewer should write word-for-word (in short phrases, abbreviating) the response of the informant. For questions asking the informant to specify names, if more than one answer is given, write all responses.

When reading questions to the informant, the interviewer should fill in the blanks with the name of the decedent. For example, "I'd like to start by asking about _____'s medical history" should be read "I'd like to start by asking about Mr. Smith's medical history. " The interviewer needs to know thoroughly the SHS definition of death to complete the interview accurately. "Death" is defined as the point at which the decedent stops breathing on his/her own and never recovers. Thus, the onset of death for someone who is resuscitated or ventilated is the point at which he/she last breathes spontaneously. He/she may recover several times after resuscitation, but the last cessation of breathing is considered "death". Death is not the time

"pronounced dead". If someone is "found dead", timing of death may be estimable if the time since last seen alive was short. However, if long, timing of death may be unknown.

The interviewer should be familiar with skip patterns and nature of each question. Several questions are similar, with only subtle differences. The interviewer must make the distinction clear to the informant. Such questions may sound repetitive and are easier if clarified.

If informant contradicts a previous answer, probe to clarify and correct if obviously wrong.

If informant says at the start of the interview that he/she does not know anything about the death, coax the informant to start the interview and try to complete. If the informant is obviously not helpful, gracefully end the interview.

Ask for next-of-kin record during the interview if appropriate but get written permission only if needed. Written release need to be witnessed.

Finally, the interviewer is responsible for reviewing and editing the Informant Interview Form thoroughly following the interview. Review every question and the skip patterns carefully. Every question must be answered unless skip patterns indicate otherwise. The description of the events preceding the death is extremely important for diagnostic purposes. Make sure that the description includes the timing of events and the symptoms experienced.

II.   DETAILED INSTRUCTIONS FOR VARIOUS QUESTIONS

ITEM DESCRIPTIONS

1-3   Information on the decedent's name, date of death, and informant should be filled out from the death certificate prior to the informant interview.

4   This question asks for the relationship of the informant to the decedent. Make sure not to reverse this: for example, "She was my mother" should be answered "daughter/son". "Other relative" includes aunt, uncle, cousin, in-law, and grandparent.

5   Ask the informant about the decedent's degree of Indian blood.

6   This question refers to any restriction from the decedent's usually day-to-day activities. It excludes the events at death.

7   "Being cared for" refers to attendant medical care because of disability or sickness.

8   Fill in as much information as is known by informant. If the informant asks why this is needed, explain that it may be important to get additional information from the nursing home, with permission, to understand the cause of death.

9   Any hospitalization for any reason is "yes".

10   Mark the appropriate answer.

11   Mark the appropriate answer.

12-13   If decedent was hospitalized more than once or stayed in more than 1 hospital, record the most recent on the form, then list all dates, names, cities and states of other hospitalizations on a separate piece of paper. If exact dates are unknown, fill in month and year. Missing values are indicated by "=" (equal sign) in the appropriate field.

14   Refer to any encounter with a physician for any reason in the month preceding death, including final symptoms.

15   This should be the most recent. If more than one physician seen, provide names and addresses of most knowledgeable two.

16   Record the name and address of decedent's "usual" physician. If same as most recently seen, record "same".

17   This question refers to chest pain from heart disease at any time before death. Angina or angina pectoris or a heart attack would be considered "yes" responses. Pain in the left arm or shoulder, jaw, or upper abdomen is considered equivalent to chest pain.

18   Refer to the list of names for nitroglycerin if informant hesitates. Nitroglycerin is usually administered as a small tablet placed under the tongue but may be taken as a pill, an ointment, or as "skin patch".

19   Be aware that this refers to past history and does not include the fatal event under consideration (emphasize ever) and clarify to the interviewee, if required.

20   Synonyms for heart attack are "myocardial infarction", "MI", coronary occlusion.

21   Coronary bypass involves surgery bypassing the blocked coronary arteries with vessels removed from the arm or leg. "Balloon dilation" or "PCTA" are other terms for angioplasty. A cardiac catheterization, coronary angiography, or angiogram for diagnostic purposes without angioplasty should be answered "no".

22   This question simply asks whether the decedent had ever had any of these cardiac events previously. Mark the appropriate response.

23   A stroke is a brain hemorrhage or ischemia (blockage of blood flow) also known as a cerebrovascular attack, cerebral hemorrhage, or blood clot on brain.

24   This includes the final, fatal event under consideration.

25   "Present" is defined as being within sight or sound of the deceased at the time of death; for example, Present: lying next to in bed, in next room and could be heard, left decedent alone momentarily. Not present: in another room out of sign and sound, outside out of sight and sound, left decedent alive and returned after 5 minutes, talked to on phone sometime right before.

26   This question asks whether anyone was present at the time of the decedent's death (defined above). If the decedent died in his/her sleep with someone nearby, Question 26 should be answered "yes".

27   Mark the shortest interval known to be reliable. If the informant hesitates, read the intervals in order starting with the shortest.

28   We are primarily interested in acute symptoms, not chronic. Thus, if a person had been generally fatigued for a month and then had chest pain one hour before death, it is the chest pain that was the last episode. Similarly, if someone had a long history of angina but, not having acute pain, suddenly collapsed and stopped breathing, the onset of the final episode was the time of collapse. If the death occurred while sleeping or while someone was within hearing range of decedent, the interval between onset and death is considered to be instantaneous. If the decedent was found dead (no one close enough to see or hear him/her), the onset may be unknown.

      Onset of last episode is defined as being at that point in time when new symptoms cause a change in activity. If the symptom is chronic (e.g. , longstanding exertional chest pain), there must be a change in severity or frequency. Symptoms might be stepwise (e.g., one chest pain, then a more severe one an hour later). In this case it is the first pain, if it was new and caused a change, that is the onset of the final episode. The final episode for someone who collapses, is revived, and collapses again began at the first collapse. Interviewers will have to probe and define onset specifically for each informant.

      The difference between Q17 and Q28 is the time period referred to. In Q28 the time is specific: within 3 days of death. In Q17, the decedent could have experienced pain at any time prior to death. If Q28 is answered "no", skip to Q32, as Q29-Q31 refer to an episode of pain within 3 days of death.

The location of the pain or discomfort referred to in Q17 and Q28 is specific. If the pain was experienced at sites other than the chest, left arm or shoulder or jaw, the answer should be "no". If the informant is unsure, but is leaning toward a "yes", then proceed as with a "yes". If the decedent was found dead, Q28 must be answered either "yes" or "unknown". If the decedent was found dead, most of the answers will be "unknown". In this case, skip quickly through, verifying that the answers are unknown.

29   The option "yes" is checked if the pain occurred anywhere in the chest within 3 days of death.

30   A list of names of "nitroglycerin" preparations is provided in the medication list and should be consulted if informant isn't sure or offers a brand name.

31   This is a crucial question for the timing of death. Use the definition provided above for death and onset of the final episode in order to clarify timing. Read the question, wait for response, and mark the shortest interval known to be true. If the informant may have given a time interval when answering Question 27. If so, the interviewer may want to preface the question stating the time interval and asking for confirmation (e.g. "You mentioned that _____ had chest pains two days before he died. Is that when the chest pain began?").

32   This question asks about any symptoms other than pain or discomfort in the chest. The timing of onset of these "other" symptoms is crucial. After each "yes" answer, probe to make sure the onset was within 3 days, and that the condition was not longstanding or"usual". Read the list slowly and fill in the appropriate answers.

33   Fill in the appropriate response.

34   Fill in as much of the information as is known.

35   This question asks if there is any person who may be able to provide additional information about the events leading up to the death or the death itself. For example, a spouse may know most about the three days prior to death while a co-worker actually witnessed the death. (Note: If the answer is "yes", an interview will need to be carried out with this individual.)

36   Fill in as much of the information as is known.

37   Write down as stated.

38-39 Narrative: Write out as close to word-for-word as possible, using short phrases. Probe neutrally for symptoms, order and timing of events, medical care, etc. Record these important items verbatim; try to limit the narrative to the space provided. When describing the events surrounding the death itself, be sure to differentiate between the onset of the last symptoms, the death (recalling definition of death), and being "pronounced dead".

40   Close the interview by thanking the informant and repeating how much the quality of our research depends on the cooperation of people like themselves. After closing the interview, fill in the questions about reliability and administrative information.

40   If informant is decedent's next of kin and agrees to provide consent for further information, ask him/her to sign the consent form.

41-44. Interviewer evaluate the quality of information provided by the informant.

THE STRONG HEART STUDY II
CARDIOVASCULAR DIEASE IN AMERICAN INDIANS

Mortality Survey --- Final Decision Form

ID number: ⬚⬚⬚⬚⬚⬚

Decedent's name: _____
| | Last | First | Middle |

Date of death: ⬚⬚ / ⬚⬚ / ⬚⬚
mo   day   yr

Age at death: ⬚⬚

Reviewer: 1=First review   2=Second review   3=Third review   9=Adjudication ⬚

A.   Cause of death, choose appropriate one. ⬚⬚

    1. Definite fatal myocardial infarction
    2. Definite sudden death due to coronary heart disease
    3. Definite fatal coronary heart disease
    4. Possible fatal coronary heart disease
    5. Definite fatal stroke
    6. Possible fatal stroke
    7. Definite fatal congestive heart failure
    8. Possible fatal congestive heart failure
    9. Other fatal cardiovascular diseases
  10. Other, specify: _____
  99. Can not be determined: _____

B.   Criteria used: (Please check the appropriate boxes)

    1.   Definite fatal myocardial infarction

        [ ] a. Definite MI within 4 weeks of death by criteria: (1=yes, 2=no)

            1. Evolving diagnostic ECG, AND/OR ⬚

            2. Diagnostic ECG and abnormal cardiac enzymes, AND/OR ⬚

            3. Prolonged cardiac pain and abnormal cardiac enzymes ⬚
      OR

        [ ] b. Acute MI diagnosed by autopsy

      AND

        [ ] c. No known non-atherosclerotic or noncardiac-atherosclerotic condition that
             was probably lethal according to death certificate, autopsy report, hospital
             records, or physician records.

2.   Definite sudden death due to CHD

[ ] a. Death witnessed as occurring within 1 hour after the onset of severe cardiac symptoms (prolonged cardiac pain, shortness of breath,fainting) or within 1 hour after the subject was last seen without symptoms.

AND

[ ] b. No documentation of acute MI within 4 weeks prior to death.

AND

[ ] c. No known non-atherosclerotic or noncardiac-atherosclerotic process that was probably lethal according to death certificate, autopsy report, hospital records, or physician report.

3.   Definite fatal CHD

[ ] a.   Death certificate with consistent underlying or immediate causes, AND
[ ] b.   No documentation of definite acute MI within 4 weeks prior to death, AND
[ ] c.   Criteria for sudden death not met (above), AND
[ ] d.   No known non-atherosclerotic or noncardiac-atherosclerotic process or event that was probably lethal according to death certificate, autopsy report, hospital records, or physician records, AND
[ ] e(i)   Previous history of MI according to relative, physician, or hospital records, or definite or possible MI by criteria, OR
[ ] e(ii)   Autopsy reporting severe atherosclerotic-coronary artery disease or old MI   without acute MI (50% proximal narrowing of two major vessels or 75% proximal narrowing of one more vessel, if anatomic details given.), OR
[ ] e(iii) Death occurring greater than 1 and less than or equal to 24 hours after the onset of severe cardiac symptoms or after subject was last seen without symptoms, OR
[ ] e(iv) Angiogram reporting greater than 1 and less than or equal to 24 hours after the onset of severe cardiac symptoms or after subject was last seen without symptoms, OR
[ ] e(v)   Other positive physical signs or lab findings.

4.   Possible fatal CHD

[ ] a.   No documentation by criteria of definite acute MI within 4 weeks prior to death, AND

[ ] b.   No documentation by criteria of definite sudden death, AND

[ ] c.   No documentation by criteria of definite fatal CHD, AND

[ ] d.   Death certificate with consistent underlying or immediate cause, AND

[ ] e. No known non-atherosclerotic or noncardiac-atherosclerotic process that was probably lethal according to death certificate, autopsy report, hospital records, or physician records.

5. Definite fatal stroke (also complete Section C)

[ ] a. Cerebral infarction or hemorrhage diagnosed at autopsy, AND

[ ] b. No other known disease process or event such as brain tumor, subdural hematoma, subarachnoid hemorrhage, metabolic disorder, or peripheral lesion that could cause localizing neurologic deficit or coma according to death certificate, autopsy, hospital records, or physician records, AND OR

[ ] c. History of rapid onset (approximately 48 hours from onset to time of admission or maximum acute neurologic deficit) of localizing neurologic deficit and/or change in state of consciousness, AND

[ ] d. Localized neurologic deficit within 6 weeks of death documented by unequivocal physician or laboratory findings with 24 hours duration of objective physician findings. AND

[ ] e. ? repeat

6. Possible fatal stroke (also complete Section C)

[ ] a. Death certificate with consistent underlying or immediate cause (ICD-9, code 431-437), but neither autopsy evidence nor adequate pre-terminal documentation of the event, AND

[ ] b. No evidence at autopsy examination of the brain, if performed, of any disease process that could cause localizing neurologic signs that would not be connected with cerebral infarction or hemorrhage.

7. Definite fatal congestive heart failure.

Two major criteria or one major and two minor criteria:

a. Major criteria

[ ] i. Paroxysmal nocturnal dyspnea or Orthopnea
[ ] ii. Neck vein distention
[ ] iii. Rales
[ ] iv. Cardiomegaly
[ ] v. Acute pulmonary edema
[ ] vi. S3 gallop
[ ] vii. Increased venous pressure > 16 cm water
[ ] viii. Circulation time ≥25 seconds
[ ] ix. Hepatojugular reflux

b. Minor criteria

[ ] i. Ankle edema
[ ] ii. Night cough
[ ] iii. Dyspnea on exertion

5.   Definite fatal stroke (also complete Section C)

[　] a.   Cerebral infarction or hemorrhage diagnosed at autopsy, AND

[　] b.   No other known disease process or event such as brain tumor, subdural
hematoma, subarachnoid   hemorrhage, metabolic disorder, or peripheral
lesion that could cause localizing neurologic deficit or coma according to
death certificate, autopsy, hospital records, or physician records,
          OR

[　] c.   History of rapid onset (approximately   48 hours from onset to time of
admission or maximum acute neurologic deficit) of localizing neurologic
deficit and/or change in state of consciousness, AND

[　] d.   Localized neurologic deficit within 6 weeks of death documented by
unequivocal physician or laboratory findings with   24 hours duration of
objective physician findings, AND

[　] e.   No other known disease process or event such as brain tumor, subdural
hematoma, subarachnoid   hemorrhage, metabolic disorder, or peripheral
lesion that could cause localizing neurologic deficit or coma according to
death certificate, autopsy, hospital records, or physician records.

6.   Possible fatal stroke (also complete Section C)

[　] a.   Death certificate with consistent underlying or immediate cause (ICD-9, code
431-437), but neither autopsy evidence nor adequate pre-terminal
documentation of the event, AND

[　] b.   No evidence at autopsy examination of the brain, if performed, of any disease
process that could cause localizing neurologic signs that would not be
connected with cerebral infarction or hemorrhage.

7.   Definite fatal congestive heart failure.

      Two major criteria or one major and two minor criteria:

   a.   Major criteria

      [　] i.   Paroxysmal nocturnal dyspnea or Orthopnea
      [　] ii.   Neck vein distention
      [　] iii.   Rales
      [　] iv.   Cardiomegaly
      [　] v.   Acute pulmonary edema
      [　] vi. S_3 gallop
      [　] vii.   Increased venous pressure > 16 cm water
      [　] viii.   Circulation time 25 seconds
      [　] ix.   Hepatojugular reflux

b.   Minor criteria

[ ] i.   Ankle edema
[ ] ii.   Night cough
[ ] iii.   Dyspnea on exertion
[ ] iv.   Hepatomegaly
[ ] v.   Vital capacity reduced by one third from maximum
[ ] vi.   Tachycardia (rate of 120/min.)

c.   Major or minor criteria

[ ] i.   Weight loss > 4.5 kg in 5 days in response to treatment

d.   [ ]   No known non-cardiac process leading to fluid overload such as renal failure

8.   Possible fatal congestive heart failure

[ ]   Death certificate with consistent underlying or immediate cause, but neither
             autopsy evidence nor adequate pre-terminal documentation of the event.

9.   Other fatal cardiovascular diseases

[ ] i.   Death certificate with consistent underlying or immediate cause.

Comment: _____

C.   TYPE OF CEREBRAL EVENT: ☐

1. Thrombo-embolic infarction   5. Other, unknown infarction
2. Subarachnoid hemorrhage   6. TIA
3. Intraparenchymal hemorrhage   7. Unknown type stroke
4. Lacunar infarction

D.   Does the diagnosis in Section A (Cause of death) agree with your clinical impression?   ☐
      1=Yes   2=No

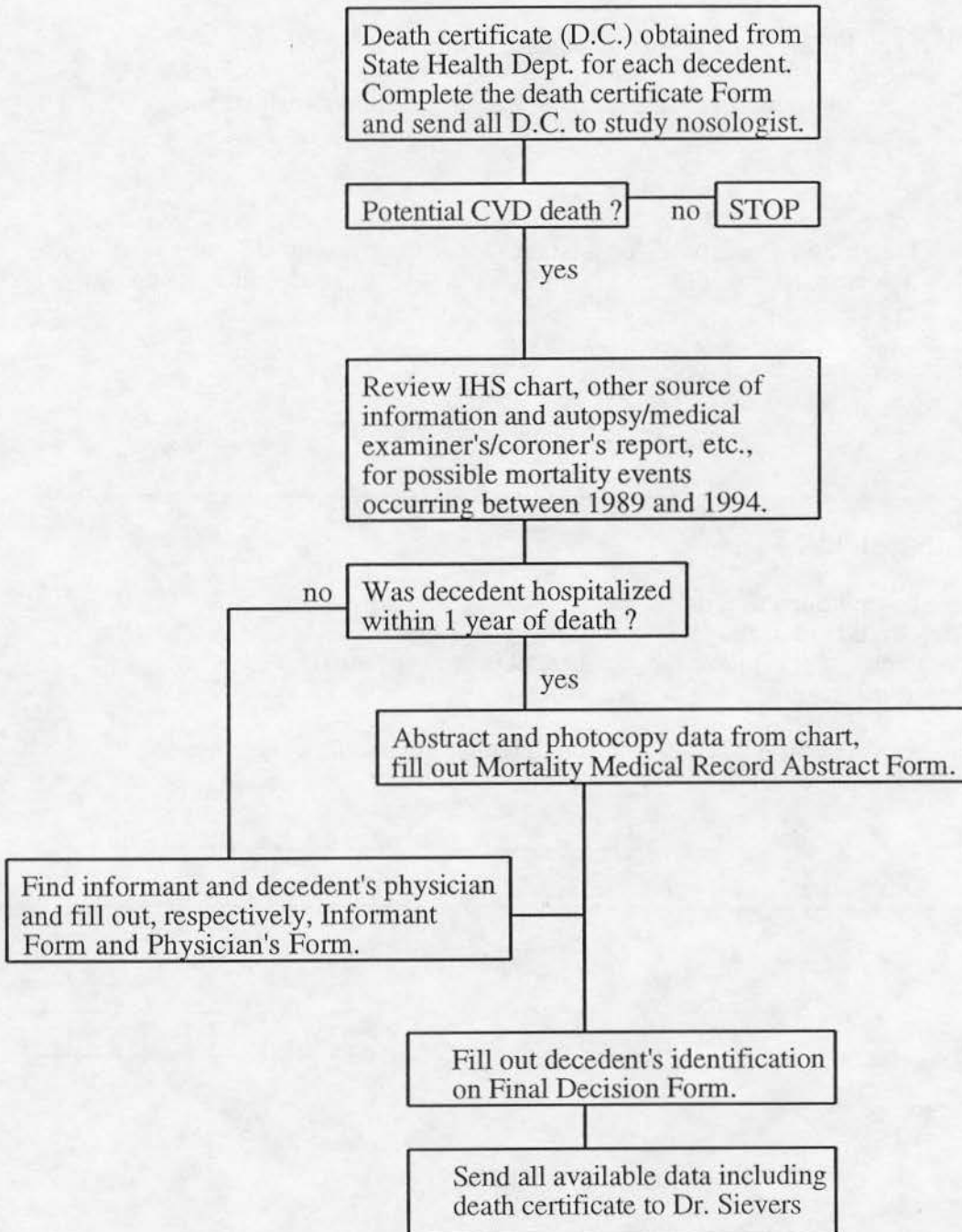If "No," what is your diagnosis? _____

Why? _____

Coder   ☐ ☐ ☐

Date completed   ☐☐/☐☐/☐☐
                                                            mo   day   yr

APPENDIX 13
THE STRONG HEART STUDY II

Flowchart for Mortality Survey

```
┌─────────────────────────────────────────┐
│ Death certificate (D.C.) obtained from   │
│ State Health Dept. for each decedent.    │
│ Complete the death certificate Form      │
│ and send all D.C. to study nosologist.   │
└─────────────────────────────────────────┘
                     │
┌──────────────────────────┐
│ Potential CVD death ?    │  no  ┌──────┐
└──────────────────────────┘      │ STOP │
                     │            └──────┘
                    yes
                     │
┌─────────────────────────────────────────┐
│ Review IHS chart, other source of        │
│ information and autopsy/medical           │
│ examiner's/coroner's report, etc.,        │
│ for possible mortality events             │
│ occurring between 1989 and 1994.          │
└─────────────────────────────────────────┘
                     │
      no  ┌──────────────────────────────┐
          │ Was decedent hospitalized    │
          │ within 1 year of death ?     │
          └──────────────────────────────┘
                     │
                    yes
                     │
┌──────────────────────────────────────────────────┐
│ Abstract and photocopy data from chart,            │
│ fill out Mortality Medical Record Abstract Form.   │
└──────────────────────────────────────────────────┘

┌──────────────────────────────────────────┐
│ Find informant and decedent's physician   │
│ and fill out, respectively, Informant      │
│ Form and Physician's Form.                 │
└──────────────────────────────────────────┘

┌──────────────────────────────────────────┐
│ Fill out decedent's identification        │
│ on Final Decision Form.                    │
└──────────────────────────────────────────┘

┌──────────────────────────────────────────┐
│ Send all available data including         │
│ death certificate to Dr. Sievers          │
└──────────────────────────────────────────┘
```

APPENDIX 14
STRONG HEART STUDY II

Checklist for Mortality Survey

ID number:

1. Death Certificate

2. ICD coded cause of death

3. Autopsy performed   yes

                                                         no

3. Autopsy report   available

                                         unavailable

4. If autopsy report is available, Autopsy Form

5. Final Decision Form I and autopsy report to Dr. Sievers

    Date: _____

6. Medical Records Abstract Form

7. Copy reports as specified

8. Check if the decedent is eligible for the morbidity survey and
    proceed as required by the morbidity survey protocol.

9. Non-IHS Physician's Form

10. Informant Interview Form?

11. Medical Records Abstract Form, Non-IHS Physician's Form
    and Informant Interview Form and Final Decision Form to
    Dr. Sievers and Dr. _____ _____

Code number of abstractor completing this form

Date completed

mo   day   yr

APPENDIX 15 (a)

SAMPLE LETTER TO INFORMANT: UNKNOWN TELEPHONE NUMBER

Dear: _____:

    I am writing on behalf of the National Heart, Lung, and Blood Institute's Strong Heart Study, a project of (name of institution) designed to measure the rates of heart disease in (name of state or area), to ask for your help. Your name is listed on the death certificate of (name of _____) who passed away on (date of the decedent). We would like to call you to talk to you about the project and to ask a few medical questions, but have been unable to find your telephone number.

    Please take a few moments to fill out and mail the enclosed postcard. The information we will be calling about will be used for statistical purposes only, and will remain strictly confidential. It will contribute to our efforts to better understand heart disease and prevent its occurrence in the future. Of course, your assistance in our research is entirely voluntary. Thank you very much in advance for your help in the important study.

                                            Sincerely,

                                            Principal Investigator

(ENCLOSE POSTCARD, RETURN ADDRESSED AND STAMPED)

APPENDIX 15 (b)

REPLY POSTCARD FROM INFORMANT WITH TELEPHONE NUMBER
FORMS SHOULD BE RETURN-ADDRESSED TO LOCAL SURVEILLANCE
CENTER AND STAMPED.

Dear (Name of Surveillance Supervisor):

     I will be able to help with your Strong Heart Study. I do have a telephone number which is
(   )   .   The best times to reach me are   or   .   An alternative telephone
number is: (   )   .   The best times to reach me at this number are   or
.

     I do not have a telephone number, but I agree to be interviewed in person, and will be
calling your staff at   (collect) to set up a time and a place for the interview.

          Sincerely,

          Print Name of Informant

APPENDIX 16

THE STRONG HEART STUDY II
CARDIOVASCULAR DISEASE IN AMERICAN INDIANS

Mortality/Morbidity Survey -- Chart Request Form

Hospital: _____   Date: _____

Chart Number	Patient Name	Date	ICD Code	Disposition

APPENDIX 17 (a)
THE STRONG HEART STUDY II
CARDIOVASCULAR DISEASE IN AMERICAN INDIANS

Morbidity Survey
Medical Records Abstract

Medical charts (IHS and/or other community hospitals) of all Phase I patients reporting a heart attack, stroke, or other vascular event will be reviewed. These events include ICD-9 codes: 402, 410 to 414, 427,428,430-438, 518.4.

ID number:

Social Security Number:

Were either of the following events diagnosed since January 1, 1989?

1. Possible Myocardial Infarction (events with codes 402, 410 to 414, 427,428, 518.4)?
    1=yes, *fill out the NEWMI form for each event*
    2=no.

2. Possible Stroke (events with codes 430-438)?
    1=yes, *fill out the NEWSTROKE form for each event*
    2=no.

IF THE ANSWERS OF 1 AND 2 ARE BOTH "NO", STOP HERE

Abstractor code

Date abstract completed

mo   day   yr

APPENDIX 17 (b)
PHOTOCOPY CHECKLIST FOR POSSIBLE MYOCARDIAL INFARCTION

Admission date: _____   ID Number: ⬚⬚⬚⬚⬚⬚

     For each hospital admission, obtaining photocopies of each of the following sections of the medical history (when available) and <u>assemble them for each admission</u>. Be sure that photocopies are legible.

	YES	NO	DONE, but Report Not Available
Admission Sheets (Face Sheets), including Diagnoses	___	___	___
Admitting History and Physical Exam	___	___	___
Discharge Summary	___	___	___
Physician's Progress Notes	___	___	___
ECGs (see instruction)	___	___	___

Procedures:

		YES	NO	DONE, but Report Not Available
1.	Echocardiogram	___	___	___
2.	Coronary angiogram	___	___	___
3.	Exercise tolerance test (Treadmill)	___	___	___
4.	Cardiac catheterization	___	___	___
5.	Coronary bypass	___	___	___
6.	Coronary angioplasty	___	___	___
7.	Swan-Ganz catheterization	___	___	___
8.	Intracoronary streptokinase, or TPA reperfusion	___	___	___
9.	Intravenous streptokinase, or TPA reperfusion	___	___	___
10.	Aortic balloon pump	___	___	___
11.	Radionuclide scan	___	___	___
12.	Other, specify: _____	___	___	___

Abstractor code   ⬚⬚⬚

Date abstract completed: ⬚⬚ / ⬚⬚ / ⬚⬚

                                                       mo   day   yr

INSTRUCTIONS FOR MORBIDITY SURVEY: MEDICAL RECORDS ABSTRACT

I. GENERAL INSTRUCTIONS

The Medical Records Abstract for the Morbidity Survey is completed for each hospitalized MI and stroke that occurred in a Phase I participant member between 1989 and 1995. The purpose of the abstract is to provide a brief summary of information related to the event, and is to used in conjunction with the associated PHOTOCOPY CHECKLISTS. The photocopies of selected materials from the medical record are used by the morbidity classification panel to determine whether the event meets the criteria for inclusion in the study. Photocopy check lists should also be used to collect information of the diagnostic tests and treatments of interest to the study (see checklists). These tests and treatments will have been identified during the medical history interview at the Phase II examination.

II. DETAILED INSTRUCTIONS FOR EACH QUESTION - FACE SHEET

Question	Instructions
1.&2.	Determine whether the patient was discharged with a diagnosis of ICD-9 codes:402, 410-414, 427, 428, 430-438, or 518.4 between January 1, 1989 and December 31, 1995. It is possible that both of these events might have occurred separately but within the time interval of interest. If this is the case, record a 1 (yes) for each item and complete both a MI and stroke form.

Separate abstracts should be completed for each event when more than one MI or more than one stroke occurred during the study interval. Begin with the first occurrence of the event in the medical record that was within the study interval. For example, if a patient had a discharge diagnosis of MI in 1989 and another in 1992, abstract the 1989 admission first. If none of the discharge diagnoses correspond to these ICD-9 codes or to the conditions covered by these codes, do not continue with the abstract. However, you may need to use the PHOTOCOPY CHECKLIST if you are collecting data on a diagnostic test or treatment procedure. If you have any questions as to whether a chart should be abstracted, contact the study coordinator. |

III. PHOTOCOPY CHECKLIST FOR POSSIBLE MI AND POSSIBLE STROKE

These instructions apply to both checklist forms.

One checklist should be used for each admission and the admission should be recorded, along with the SHS ID number. For each item on the list, indicate whether or not it was done/is available (e.g., discharge summary). If a procedure was done but the report is not in the chart, check the last column. For those items marked "yes", a photocopy should be attached. Be sure that personal identifiers are removed from the report, but that the SHS ID is printed on each page that is photocopied. **IT IS VERY IMPORTANT THAT THE XEROX BE LEGIBLE** since these documents will be reviewed by the SHS morbidity panel. Compile all documents for a single admission, use the checklist as a cover sheet, and forward the packet to the coordinating center.

Enter your code number and the date the checklist was completed.

APPENDIX 17 (c)
THE STRONG HEART STUDY II
CARDIOVASCULAR DISEASE IN AMERICAN INDIANS

Morbidity Survey
Medical Records Abstract for Myocardial Infarction

ID number: ⬚⬚⬚⬚⬚

1. a. Last Hospital code number ⬚⬚⬚⬚⬚

    b. Hospital name _____

    c. Hospital location _____

    d. Medical record number ⬚⬚⬚⬚⬚⬚⬚⬚⬚⬚

2. Date of ADMISSION to this hospital:

    ⬚⬚/⬚⬚/⬚⬚
    mo   day   yr

3. Date of discharge:

    ⬚⬚/⬚⬚/⬚⬚
    mo   day   yr

4. Was the patient transferred to or from another acute care hospital? ⬚
    (1=yes,   2=no)
    If no, go to Question 6.

5. Care received for heart attack and/or stroke or other problem. *List all facilities where patient was hospitalized since January 1, 1989.*

    Reason: 1=Heart attack   2=Stroke   3=Other, please specify.

	Hospital/Clinic	Town/State	Date (mo/day/yr)	Reason
i.	_____	_____	__/__/__	⬚
ii.	_____	_____	__/__/__	⬚
iii.	_____	_____	__/__/__	⬚
iv.	_____	_____	__/__/__	⬚
v.	_____	_____	__/__/__	⬚
vi.	_____	_____	__/__/__	⬚
vii.	_____	_____	__/__/__	⬚
viii.	_____	_____	__/__/__	⬚
ix.	_____	_____	__/__/__	⬚
x.	_____	_____	__/__/__	⬚

6.   Enter the ICD-9 code numbers for the hospital discharge diagnoses and procedure codes recorded in the medical record exactly as they appear on the front sheet of the medical record and/or on the discharge summary. Be sure they are ICD-9 codes. Record diagnoses if no codes are available.

1. ☐☐☐ • ☐☐   6. ☐☐☐ • ☐☐

2. ☐☐☐ • ☐☐   7. ☐☐☐ • ☐☐

3. ☐☐☐ • ☐☐   8. ☐☐☐ • ☐☐

4. ☐☐☐ • ☐☐   9. ☐☐☐ • ☐☐

5. ☐☐☐ • ☐☐   10. ☐☐☐ • ☐☐

7.   Were any cardiac enzymes reported within DAYS 1-4 after arrival at the hospital or after in-hospital CHD event ?   (1=yes, 2=no)   ☐

IF CARDIAC ENZYME TESTS WERE DONE, COMPLETE THE ENZYME TEST RESULTS IN THE FOLLOWING PAGE

8.   Were any 12 lead ECG's taken during this admission?   (1=yes,   2=no)   ☐

If ECGs were taken, attach copies of required ECGs and interpretations, at least one per day. (see instructions)

9.   Abstractor Number

10.   Date abstract completed

mo   day   yr

CARDIAC ENZYME TEST RECORDS

Hospital Admission Date: _____/_____/_____ Complete Separate Forms for Each Admission in Which Enzyme Were Done.

SPECIFY THE UNIT OF MEASUREMENT	DAY ONE (DATE:)			DAY TWO (DATE:)			DAY THREE (DATE:)			DAY FOUR (DATE:)		
	No.1	No.2	No.3	No.1	No.2	No.3	No.1	No.2	No.3	No.1	No.2	No.3
Total CK (CPK)												
Upper Limit of Total CK (CPK)												
CK-MB												
Upper Limit of CK-MB												
Total LDH												
Upper Limit of Normal LDH												
LDH1												
LDH2												
LDH1/LDh2												
SGOT												
Upper Limit of Normal SGOT												

DETAILED INSTRUCTIONS FOR EACH QUESTION
- NEWMI ABSTRACT

Question	Instructions

Enter the SHS ID number.

1. Enter the hospital code number, name, city and state and the medical record number of the chart in the appropriate boxes.

2. Enter the date of admission for the event you are abstracting.

3. Enter the date of discharge from the hospital for the event you are abstracting.

4. If the patient was transferred to or from another acute care hospital, record a 1 in the code box. This question asks only about acute care facilities, and does not include convalescent care, long-term custodial care, or outpatient clinics or physicians' offices.

5. Question 5 is designed to be sure that information is collected on all events of interest that have occurred since the Phase I exam. This listing will serve to keep track of which records need to be obtained for any given participant. Record the location, date and reason for all hospitalizations since January 1, 1989. These are only to include hospitalizations for coronary diseases or stroke.

6. Enter the ICD-9 codes of the discharge diagnoses and procedure codes as listed on the front sheet of the discharge summary. Record them in the order they are listed in the chart. Be sure these are ICD codes and not another coding system such as APS. If the codes are not ICD-9, leave the boxes blank but print the diagnoses in the space provided.

7. Determine whether any cardiac enzyme studies were done within 1-4 days after arrival at this hospital or after an in-hospital CHD event. Cardiac enzymes include CK (CPK), LDH, and SGOT.

    If cardiac enzyme studies were done within the appropriate time frame after the event, complete the Enzyme Test Form. Include the hospital admission date and the dates of each test. You must specify THE UNIT OF MEASUREMENT (e.g., mg/dl, ug, international units (IU), etc.). The UPPER LIMIT OF NORMAL for each enzyme must also be included. If it is not included on the lab report, you must contact the lab to obtain the limit values at the time the test was done. THE UPPER LIMIT BOXES CANNOT BE LEFT BLANK.

8. Code as instructed. If yes, attach copies of required ECGs and interpretations. These include: the first ECG recorded after admission or the occurrence of an in-hospital event, the first ECG done each day thereafter, and the last ECG recorded before discharge. Be sure that personal identifiers are removed, but also that the SHS ID is recorded on each ECG attached. ECGs should also be dated so that it is possible to determine the order in which they were done. (ECGs are also included on the PHOTOCOPY CHECKLIST.)

9. Enter your code number.

10. Enter the date the abstract was completed.

APPENDIX 17 (d)
PHOTOCOPY CHECKLIST FOR NEW STROKE

Admission date _____   ID number: [   ]

For each hospital admission, obtaining photocopies of each of the following sections of the medical history (when available) and assemble them for each admission. Be sure that photocopies are legible.

	YES	NO	DONE, but Report Not Available
Admission Sheets (Face Sheets), including diagnoses	___	___	___
Admitting History and Physical Exam	___	___	___
Discharge Summary	___	___	___
Physician's Progress Notes	___	___	___
Neurology Consult Report	___	___	___

Reports of Procedures of:

		YES	NO	DONE, but Report Not Available
1.	Computerized Axis Tomography (CAT or CT) of the head	___	___	___
2.	Magnetic Resonance Image (MRI) of the head	___	___	___
3.	Carotid ultrasound/doppler	___	___	___
4.	Lumbar puncture	___	___	___
5.	Electrocardiogram	___	___	___
6.	Angiography	___	___	___
7.	Other, specify: _____	___	___	___

Abstractor code   [   ][   ][   ]

Date abstract completed   [   ][   ] / [   ][   ] / [   ][   ]

                                                            mo   day   yr

THE STRONG HEART STUDY II
CARDIOVASCULAR DISEASE IN AMERICAN INDIANS

Morbidity Survey
Medical Records Abstract for Stroke

ID number: ☐☐☐☐☐☐

1. a. Last Hospital code number ☐☐☐☐☐☐

    b. Hospital name _____

    c. Hospital location _____

    d. Medical record number ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

2. Date of ADMISSION to this hospital: ☐☐ / ☐☐ / ☐☐
    mo day yr

3. Date of discharge: ☐☐ / ☐☐ / ☐☐
    mo day yr

4. Was the patient transferred to or from another acute care hospital? ☐
    (1=yes, 2=no) *If no, go to Question 6.*

5. Care received for heart attack and/or stroke or other problem. List all
    facilities where patients was hospitalized since January 1, 1989.

    Reason: 1=Heart attack 2=Stroke 3=Other, please specify.

	Hospital/Clinic	Town/State	Date(mo/day/yr)	Reason
i.	_____	_____	__/__/__	☐
ii.	_____	_____	__/__/__	☐
iii.	_____	_____	__/__/__	☐
iv.	_____	_____	__/__/__	☐
v.	_____	_____	__/__/__	☐
vi.	_____	_____	__/__/__	☐
vii.	_____	_____	__/__/__	☐
viii.	_____	_____	__/__/__	☐
ix.	_____	_____	__/__/__	☐
x.	_____	_____	__/__/__	☐

6. Enter the ICD-9 code numbers for the hospital discharge diagnoses recorded in all medical records and procedure codes exactly as they appear on the front sheet of the medical record and/or on the discharge summaries. Be sure they are ICD-9 codes. Record diagnoses if no codes are available.

1. ☐☐☐☐ • ☐☐   6. ☐☐☐☐ • ☐☐

2. ☐☐☐☐ • ☐☐   7. ☐☐☐☐ • ☐☐

3. ☐☐☐☐ • ☐☐   8. ☐☐☐☐ • ☐☐

4. ☐☐☐☐ • ☐☐   9. ☐☐☐☐ • ☐☐

5. ☐☐☐☐ • ☐☐   10. ☐☐☐☐ • ☐☐

7. Abstractor code

8. Date abstract completed

☐☐ / ☐☐ / ☐☐
mo   day   yr

DETAILED INSTRUCTIONS FOR EACH QUESTION - NEWSTROKE

Question   Instructions

Enter the SHS ID number.

1.   Enter the hospital code number, name, city and state and the medical record number of the chart in the appropriate boxes.

2.   Enter the date of admission for the event you are abstracting.

3.   Enter the date of discharge from the hospital for the event you are abstracting.

4.   If the patient was transferred to or from another acute care hospital, record a 1 in the code box. This question asks only about acute care facilities, and does not include convalescent care, long-term custodial care, or outpatient clinics or physicians' offices.

5.   Question 5 is designed to be sure that information is collected on all events of interest that have occurred since the Phase I exam. This listing will serve to keep track of which records need to be obtained for any given participant. Record the location, date and reason for all hospitalizations since January 1, 1989. These are only to include hospitalizations for coronary diseases or stroke.

6.   Enter the ICD-9 codes of the discharge diagnoses and procedure codes as listed on the front sheet of the discharge summary. Record them in the order they are listed in the chart. Be sure these are ICD codes and not another coding system such as APS. If the codes are not ICD-9, leave the boxes blank but print the diagnoses in the space provided.

7.   Enter your code number.

8.   Enter the date the abstract was completed.

APPENDIX 17 (f)

THE STRONG HEART STUDY II
CARDIOVASCULAR DISEASE IN AMERICAN INDIANS

Morbidity Survey
Possible Myocardial Infarction
ECG Analysis

ECG CENTER SHEET

High Resolution photocopies of ECGs taken as evidence of a myocardial infarction during the morbidity survey should be arranged in chronological order from earliest to latest. ECG series for each case will be reviewed independently by three cardiologists at the ECG Reading Center (Fitzsimons). When possible, a baseline ECG obtained most recently, but prior to the event in question, should be labeled "BASELINE"and included as the top tracing.

ID number:

1) ECG READER ID number:

The series of ECGs will be assigned the highest category for which criteria are met, i.e., evolving diagnostic diagnostic equivocal other.

EVOLVING DIAGNOSTIC

Definition: An evolving pattern on serial ECGs of a diagnostic ECG. (An evolving pattern of changes [appearance or disappearance within lead groups: anterior (V1 -V5); lateral (I, aV L , V6); or inferior (II, III, aV F)] establishes the infarct as acute. Two or more ECG recordings during the hospitalization are needed for this classification.)

To Qualify as a Q wave, deflection should be at least 0.1 mV (1 mm.) in amplitude.

Possibilities:

☐ No Q wave in one ECG record followed by a record with a diagnostic Q wave.

OR

☐ An equivocal Q wave and no major ST segment depression in one ECG followed by a record with a diagnostic Q wave PLUS a major ST segment depression.

OR

☐ An equivocal Q wave and no ST segment elevation in one ECG record followed by a record with a diagnostic Q wave PLUS ST segment elevation than or = to 1 mm.

OR

☐ An equivocal Q wave and no major T wave inversion in one ECG record followed by a record with a diagnostic Q wave PLUS a major T wave inversion.

OR

☐ No Q wave and no ST Junction depression than or = to 0.5 mm and flat or downsloping ST segment depression followed by a record with an equivocal Q wave PLUS ST Junction and flat or downsloping ST depression of than or = to 0.5 mm.

OR

☐ No Q wave and no ST elevation than or = to 1 mm. followed by a record with an equivocal Q wave PLUS ST elevation or = to 1 mm.

OR

☐ No Q wave and no T wave findings diagnostic of infarction followed by a record with an equivocal Q wave PLUS T wave findings diagnostic of infarction.

DIAGNOSTIC ECG WITH Q WAVE

☐ Diagnostic Q and QS patterns.

DIAGNOSTIC ECG WITHOUT Q WAVE

☐ ST segment elevation PLUS T wave depression indicative of infarction. (T wave depression cannot be used in the presence of ventricular conduction defects.)

EQUIVOCAL ECG WITH Q WAVE

☐ ECG with Q and QS pattern possibly representing infarction.

EQUIVOCAL ECG WITHOUT Q WAVE

☐ ST junction (J) and segment depression or T wave inversions or ST segment elevations possibly representing infarction.

OTHER

☐ All other findings, including normal.

UNCODEABLE ECG

☐ 1) Missing Leads

2) Baseline drift (1 in 20) if it obscures ST-T segment.

3) Muscle tremor giving 2 mm peak-to-peak oscillation.

4) Other technical errors making Q wave measurements impossible

5) Major abnormal QRS conduction patterns (BBB, pacer, etc.)

THE STRONG HEART STUDY II

Morbidity Survey --- DECISION FORM

ID number:

Participant's name: _____

Last   *First*   *Middle*

Date of this event

mo   day   yr

Deposition: 1=Regular   2=QC   3=Equivocal   9=Adjudication

A.   DIAGNOSIS (enter appropriate code number):

1.   Definite non-fatal myocardial infarction
2.   Possible non-fatal myocardial infarction
3.   Definite non-fatal stroke
4.   Possible non-fatal stroke
6.   Definite CHD
7.   Possible CHD
8.   TIA
9.   Other CVD, specify: _____
10.   Non -CVD, specify:
_____

B.   Criteria used: (Please check one box in each field)

1.   MYOCARDIAL INFARCTION

a.   PROLONGED CARDIAC PAIN   Present

Absent

b.   ECG FINDINGS   Evolving diagnostic ECG

Diagnostic ECG

Equivocal ECG

Absent, uncodable, or other

c.   CARDIAC ENZYMES   Abnormal

Equivocal

Incomplete

Normal

COMMENTS: _____

_____

_____

_____

2.   STROKE

    a.   DIAGNOSTIC EVIDENCE

        Unequivocal physician or laboratory   ☐

        Discharge diagnoses of stroke (431, 432, 434, 436, 437)   ☐

        Neither of above   ☐

    b.   ONSET/DURATION OF NEUROLOGICAL DEFICIT

        Rapid/ > 24 hours   ☐

        Rapid/ < 24 hours   ☐

        Protracted/ > 24 hours   ☐

        Protracted/ < 24 hours   ☐

    c.   OTHER CAUSES   Present   ☐

                         Absent   ☐

    d.   TYPE OF STROKE: ☐

1.	Thrombo-embolic infarction	5.	TIA
2.	Subarachnoid hemorrhage	6.	Other, unknown infarction
3.	Intraparenchymal hemorrhage	7.	Unknown type stroke
4.	Lacunar infarction		

COMMENTS: _____

_____

_____

_____

3. Coronary Heart Disease (CHD)

    a. Cardiac cath proven coronary artery disease ☐

    b. PTCA ☐

    c. Coronary artery bypass grafting ☐

    d. Abnormal stress ECG ☐

    e. Abnormal imaging ☐

    f. Positive functional test of ischemia (such as treadmill) ☐

COMMENTS: _____

_____

_____

_____

4. Other Non-fatal Cardiovascular Disease

    a. Congestive Heart Failure ☐

    b. Cardiomyopathy ☐

    c. Valvular Heart Disease ☐

    d. Left ventricular Hypertrophy ☐

    e. Atrial Fibrillation ☐

    f. Noncoronary heart surgery or carotid or other vascular surgery ☐

    g. Pacemaker implantation ☐

    h. Positive non-coronary angiography ☐

COMMENTS: _____

_____

_____

_____

C.   Does the diagnosis in Section A (DIAGNOSIS) agree with your clinical impression?   ☐
            1=Yes   2=No

      If "No", what is your diagnosis? (Diagnosis in A)   ☐

      Why? _____

            _____

            _____
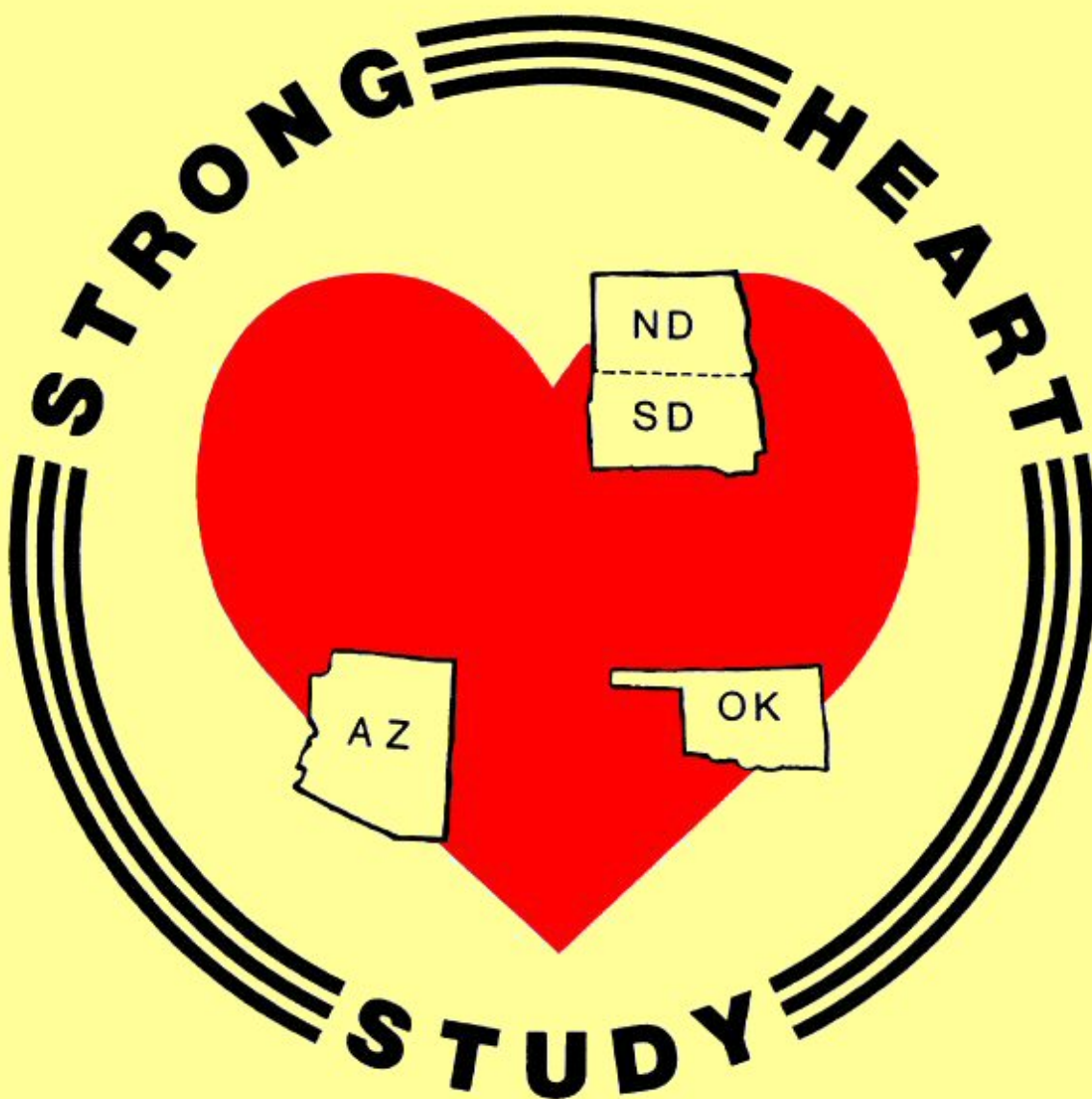
Coder   ☐☐☐

Date completed: ☐☐／☐☐／☐☐
                                                                                        mo   day   yr

**CARDIOVASCULAR DISEASE IN AMERICAN INDIANS
(PHASE II)**

OPERATIONS MANUAL - VOLUME TWO

PERSONAL INTERVIEW AND GENERAL EXAMINATION

THE NATIONAL HEART, LUNG AND BLOOD INSTITUTE
OF THE NATIONAL INSTITUTES OF HEALTH

THE STRONG HEART STUDY

Cardiovascular Disease in American Indians
(Phase II)

Operational Manual

Volume Two

Personal Interview and General Examination

July 1, 1993

VOLUME II
PERSONAL INTERVIEW AND GENERAL EXAMINATION

Table of Contents

1. Clinical Examination - General

1.1 INTRODUCTION

Tribal members who have resided in one of the study communities for at least 6 months and who are between 45 and 74 years of age during the examination phase were invited to participate in the Phase I physical examination. Persons who are institutionalized were excluded. All those who participated in the Phase I exam are eligible for the Phase II exam. This component of the study consists of a personal interview, a limited physical examination and laboratory tests. The objectives are to estimate the prevalence of CVD and its risk factors and to assess the degree of association between the risk factors and CVD.

The examination will be conducted at local IHS hospitals and clinics. In the Dakotas, it will be performed at the Aberdeen Area IHS hospitals and clinics on three reservations. In Phoenix, the IHS hospital at Sacoton (GRIC), the IHS outpatient clinic at Salt River (SRIC) and the outpatient clinical AkChin will be the examination sites. In Oklahoma, the IHS hospital in Lawton and the IHS clinic in Anadarko will provide space and facilities for the examination.

The objective of the Strong Heart Study and the examination procedures will be explained to the participants. A consent form will be signed by each participant. Appendix 1 gives an example of the consent form.

All examinations are performed by trained personnel, nurse practitioners, registered nurses, medical students, physician assistants or physicians. All examination items are within the scope of training that these providers have received and are usual, if not daily, parts of physical examinations. Detailed descriptions and training are aimed at achieving consistency from examination to examination, and among centers. This is the main goal of this component of the protocol.

The training of the registered nurses, nurse practitioners, medical students, physician assistants and physicians on the Phase II protocol occurred on April 22, 1989 at the Black Hills Training Center, Rapid City, S.D. and is based on the written protocol. Each Study Center has designated a primary examiner and at least one other person who is available to perform examinations in the absence of this primary person.

Certification requires adequate performance of the components of the examination as validated during training. In case of loss of a center's staff member, a replacement may be trained locally by someone certified in the procedure(s). The same certification requirements as used in the initial training must be met. Quality control focuses on the potential for false positive examinations. Because most participants are healthy, the frequency of abnormal findings is relatively small. The presence of real abnormalities

among those with normal examinations is also small (a low false negative rate), and this makes it inefficient to re-examine the many individuals with normal findings. The review of positive findings is part of the medical data review. After the initial training, continuing education includes regular review of the protocol.

1.2 COMPONENTS OF THE CLINICAL EXAMINATION, ENDPOINTS AND RISK FACTORS

1.2.1 Components of the Clinical Examination

The clinical examination has two parts: a personal interview and a physical examination.

1.   Personal Interview

The following questionnaires will be administered.

(1)   Demographic information, personal habits including smoking, alcohol and beverage consumption, stress and acculturation.

(2)   Medical history, including Rose questionnaire for angina pectoris and intermittent claudication

(3)   Dietary survey

2.   Physical Examination

The physical examination includes the following procedures:

(1)   Anthropometric measurements
     (a)   Weight
     (b)   Height
     (c)   Waist and hip circumferences
     (d)   Body fat measurement using an impedance meter
     (e)   Arm circumference (for blood pressure measurements)

(2)   Examination of the following
     (a)   Lungs
     (b)   Pulses - posterior tibial and dorsalis pedal
     (c)   Bruits - Carotid and femoral

(3)   Blood Pressure Measurements:
     (a)   Sitting with conventional sphygmomanometer(3 times) -right arm
     (b)   Right brachial and both ankles using doppler in supine position

(4)   Twelve-lead ECG measurement

(5)   Glucose Tolerance Test (GTT). The GTT will be given to participants excluding the following:

      (a)   Insulin requiring diabetics
      (b)   Diabetics who are on oral agents and the previous record indicated at least two values of random blood glucose above 250 mg/dl, or fasting glucose 225 mg/dl. by One Touch.
      (c)   Non-diabetics with a fasting glucose 225 mg/dl. by One Touch.

(6)   Fasting blood samples for measurements of total triglyceride and cholesterol, LDL and HDL Cholesterol, VLDL Cholesterol and TG, Plasma Fibrinogen, and PAI-1, RBC type, and DNA isolation, glucose, creatinine, insulin, and HbA1c. No fasting blood samples will be taken from patients who are on renal dialysis or have had a kidney transplant, except tubes for isolation of DNA assay and RBC type.

(7)   Urine collection at beginning of physical examination for measurement of albumin and creatinine.

(8)   Peripheral sensitivity as measured by monofilaments.

(9)   Echocardiography

(10)   Gallbladder ultrasound

(11)   Pulmonary function test

(12)   Tuberculin skin testing

(13)   Coccidiomycosis skin testing (Arizona only)

The IHS medical records will also be reviewed to determine whether the participant experienced hospitalization for stroke, myocardial infraction, in 1988-94.

A checklist to be used for the physical examination and a reminder of post examination activities are given in Appendix 2 (a) and (b).

The clinical examination will last approximately three hours. The participant will arrive at the clinic fasting in the morning. After registration, a study staff member will explain the study and procedures to the participant, answer questions, if any, and have the

consent form signed. The participant will then be instructed to go to the laboratory for blood drawing, to drink the glucose preparation (Glutol), and to obtain the urine specimen. The nurse clinician and other staff will then conduct the personal interview, examination of the lungs, obtain anthropometric measurements, blood pressure, impedance measurement for body fat composition, and ECG measurements. At exactly two hours after the ingestion of the glucose preparation, the participant will have another blood sample drawn for the glucose tolerance test. If the above procedures are not completed before the 2-hour sample is drawn, they may be continued and completed after the participant consumes a light snack. After all the procedures are completed, the participant will receive the payment or sign the payment form and be thanked for his/her participation. A flowchart that describes the process is given in Appendix 2 (c).

If possible, all of the components, except for the dietary survey and echo exams, should be completed in one visit. If an individual leaves before the examination is completed, it must be completed before the study is completed. The personal interview and consent may be completed up to two weeks prior to the physical examination if such arrangements are more convenient.

1.2.2 Endpoints and Risk Factors

A. MORBIDITY EVENT CRITERIA

1. Definite Myocardial Infarction (MI)

Minnesota codes 1.1.x or 1.2.x except 1.26. and 1.28 with no 7.1 or 7.4
History of MI verified by chart review as definite MI

2. Possible Myocardial Infarction

Minnesota codes 1.3.x, 1.2.6, or 1.2.8 with no 7.1 or 7.4
History of MI verified by chart review as possible MI

3. Definite Coronary Heart Disease (CHD)

Definite MI,
Definite CHD verified by chart review to include cardiac cath, proven coronary artery disease, PTCA, coronary artery bypass grafting, or abnormal stress ECG plus abnormal imaging (i.e., both must be abnormal),
Angina Pectoris plus LBBB (7.1.1) or
                      ST changes (4.1) or
                      T wave changes (5.1) or
                      verified possible MI,

4.   Possible Coronary Heart Disease

       Possible ECG MI (1.3.x, 1.2.6, 1.2.8)
       Angina Pectoris
       Minnesota codes 7.1, 4.1, 4.2, 5.1, 5.2, 7.4
       Unconfirmed history of MI
       Positive functional test of ischemia (such as treadmill) without invasive confirmation
       Possible ECG <u>or</u> imaging in scintigraphic studies (not both).

5.   Definite Cardiovascular Disease (CVD)

       Definite CHD
       Congestive Heart Failure
       Cardiomyopathy
       Valvular Heart Disease
       Left ventricular Hypertrophy by Echocardiogram
       Left ventricular Hypertrophy by ECG (3.1 or 3.3 <u>plus</u> 4.1-4.3 or 5.1-5.3)
       Ankle Arm Index <= 0.8
       Atrial Fibrillation
       Minnesota codes 4.1, 5.1, 6.1, 6.2, 6.8, 7.1, 7.2, 7.4
       Noncoronary heart surgery or carotid or other vascular surgery
       Pacemaker implantation
       Bruits by physical examination
       Intermittent Claudication by Rose Questionnaire
       Positive non-coronary angiography

DEFINITION OF ANGINA PECTORIS AND HISTORY OF MI:

1.   ANGINA PECTORIS - DEFINED BY THE ROSE QUESTIONNAIRE:

       ROSEAP=1 (YES):   ROSE1='1' AND (ROSE2='1' OR ROSE2='3') AND ROSE4='1'
                         AND ROSE5='1' AND ROSE6='1' AND (ROSE7A='1' OR
                         ROSE7B='1' OR (ROSE7C='1' AND ROSE7D='1')), ELSE
       ROSEAP=0 (NO)

2.   MYOCARDIAL INFARCTION

       A.   MEDICAL HISTORY
              1.   HISTORY OF MI: Q3l IN MEDICAL HISTORY QUESTIONNAIRE
                     MED37='1';
              2.   POSSIBLE MI FROM ROSE QUESTIONNAIRE: Q9
                     ROSE9='1'.

B. **DEFINITION OF HYPERTENSION**

1. BLOOD PRESSURE: AVERAGE OF THE LAST TWO SITTING BLOOD PRESSURE
   FROM PHYSICAL EXAM, Q17, Q18, Q19, AND Q20
   SYSTOLIC BLOOD PRESSURE -   SBP = (EXAM27 + EXAM29) / 2
   DIASTOLIC BLOOD PRESSURE - DBP = (EXAM28 + EXAM30) / 2
   MEAN BLOOD PRESSURE -MBP = (2/3 SBP) + (1/3 DBP)

2. HYPERTENSION

   a. WHO CRITERIA

   HYPERTENSION:
   i.   TAKING ANTIHYPERTENSIVE DRUG(MEDICATION CODE='2408')
         OR
   ii.   TAKING (DIURETICS ('4028'), OR BETA-BLOCKERS ('1216')) AND
         HISTORY OF HYPERTENSION (MED19='1')
         OR
   iii. SYSTOLIC BLOOD PRESSURE $\geq$ 160 mmhg
         OR
   iv.   DIASTOLIC BLOOD PRESSURE $\geq$ 95 mmHg

   BORDERLINE HYPERTENSION:
   140 mmHg $\leq$ SBP < 165 mmHg OR
   90 mmHg $\leq$ DBP < 95 mmhg

   NORMOTENSIVE:
   SBP < 140 AND DBP < 90 AND NO ANTIHYPERTENSIVE
   TREATMENT.

   b. US CRITERIA:
   HYPERTENSION: WHO HYPERTENSION OR BORDERLINE
                   HYPERTENSION
   NORMOTENSIVE: SAME AS WHO NORMOTENSIVE.

HYPERTENSION CONTROL, FOR HYPERTENSIVE PARTICIPANTS ONLY:

1. UNCONTROLLED HYPERTENSION: DBP $\geq$ 90 OR SBP $\geq$ 140

C. **DEFINITION OF RENAL DISEASE:**

1. RENAL FUNCTION, PLASMA CREATININE:

a. CATEGORICAL VARIABLE:
   1 (RENAL INSUFFICIENCY)   PLASMA CREATININE $\geq$ 2.0 mg/dl
   0 (NORMAL)   PLASMA CREATININE < 2.0 mg/dl
b. CONTINUOUS VARIABLE, ADJUSTED FOR BMI

2. ALBUMINURIA (ACRATIO):
ESTIMATED BY URINARY ALBUMIN - URINARY CREATININE RATIO
2 (MACROALBUMINURIA)   ACRATIO $\geq$ 300 mg/g
1 (MICROALBUMINURIA)   ACRATIO 30 - 299 mg/g
0 (NORMAL)   ACRATIO < 30 mg/g

3. END STAGE RENAL DISEASE (ESRD)
1 (YES)=   ON RENAL DIALYSIS, MEDICAL HISTORY FORM, Q4a, MED42='1', OR
    HAD KIDNEY TRANSPLANT, MEDICAL HISTORY, Q4b, MED43='1', OR
    KIDNEY FAILURE, MEDICAL HISTORY, Q3g, MED29='1'
0 (NO)=   NONE OF ABOVE

D. DEFINITION OF PERIPHERAL VASCULAR DISEASE (PVD)

1. ANKLE-BRACHIAL RATIO (PVD_ABR), PHYSICAL EXAM, Q44, Q45, AND Q46
    RIGHT ANKLE BP: MEAN OF FIRST AND SECOND DOPPLER SBP OF RT ANKLE.
    RANKBP=(EXAM66 + EXAM68) / 2
    LEFT ANKLE BP: MEAN OF FIRST AND SECOND DOPPLER SBP OF LT ANKLE.
    LANKBP=(EXAM70 + EXAM72) / 2
    RIGHT ARMBP: MEAN OF FIRST AND SECOND DOPPLER SBP OF RT ARM.
    RARMBP=(EXAM74 + EXAM75) / 2
    RPVD_ABR = RANKBP / RARMBP
    LPVD_ABR = LANKBP / RARMBP

PVD_ABR:
1 (YES):   IF (RPVD_ABR < 0.8) OR (LPVD_ABR < 0.8) OR THE ANKLE DOPPLER BPs WERE NOT AUDIBLE (EXAM70, EXAM72, EXAM74, OR EXAM75 WAS '0')
0 (NO):   IF PVD_ABR $\geq$ 0.8.

2. PERIPHERAL OCCLUSION (PERIOCC):
ABSENCE OF DORSALIS PEDIS PULSE **AND** POSTERIOR TIBIAL PULSE ON EITHER FOOT.

(PHYSICAL EXAM Q36-Q39),
PERIOCC=1 (YES): (EXAM58='2' AND EXAM60='2') OR (EXAM59='2' AND
                  EXAM61='2')
PERIOCC=0 (NO):   EXAM58='1' AND EXAM59='1' AND EXAM60='1' AND
                  EXAM61='1'

3.   PRESENCE OF FEMORAL BRUITS (BRUIT)
      (PHYSICAL EXAM Q40-Q41)
      BRUIT=1 (YES):   EXAM62='1' OR EXAM63='1'
      BRUIT=0 (NO):   EXAM62='2' AND EXAM63='2'

4.   INTERMITTENT CLAUDICATION (MEDICAL HISTORY - ROSE QUESTIONNAIRE)
      ROSEIC=1 (YES):   ROSE10='1' AND ROSE11='1' AND ROSE12='1' AND
                        (ROSE13='1' OR ROSE13='3') AND ROSE15='2' AND
                        ROSE16='1' AND ROSE17='1' AND ROSE18='1', ELSE
      ROSEIC=0 (NO):

5.   COMPOSITE PVD (PVD_COMP)
      PVD_COMP 1 (YES):PVD_ABR=1 OR PERIOCC=1 OR BRUIT=1 OR ROSEIC=1
      PVD_COMP 0 (NO): PVD_ABR=0 AND PERIOCC=0 AND BRUIT=0 AND
      ROSEIC=0

E.   **DEFINITION OF OBESITY INDICES, PHYSICAL EXAM:**

a.   BODY MASS INDEX, Q1 AND Q2, (WEIGHT IN KILOGRAM) / (HEIGHT IN
      METER)^2

         BMI = (EXAM94) / (EXAM03/100)^2

b.   WAIST-HIP RATIO, Q33 AND Q9:

         WHR = EXAM51 / EXAM13

c.   PERCENT BODY FAT (PCTFAT):

      PCTFAT is calculated by using Rising's equation as following:

      fat-free mass:
      FFT = 13.74 + 0.25 * (height^2 / resistance) + 0.30 * (weight) - 0.14 * (age) + 6.18 *
      (sex)
         where: height in cm, weight in kg, age in years, sex (0=female, 1=male)

      fat mass (FM) = weight - FFT

PCTFAT = (FM / weight) * 100%

RESISTANCE: Q35a IN PHYSICAL EXAM

1.2.2 DEFINITION OF RISK FACTORS

1.   CIGARETTE SMOKING (PERSONAL INTERVIEW II, Q24-Q29):

    A.   SMOKING (NEVER, EX-SMOKER, CURRENT)

0 (NEVER)	IF INT2_34='2' OR INT2_35=0
1 (EX-)	IF (INT2_34 = '1' AND INT2_35 NE 0) AND INT2_36='2'
2 (CURRENT)	IF (INT2_34 = '1' AND INT2_35 NE 0) AND INT2_36='1'
9 (UNKNOWN)	NONE OF ABOVE

IF GROUP INTO SMOKER VS NONSMOKER,
(SMOKING=0 OR SMOKING=1) CAN BE COMBINED AS NON-CURRENT SMOKER;

OR

(SMOKING=1 OR SMOKING=2) CAN BE COMBINED AS EVER SMOKED.

    B.   SMOKING AMOUNT (FOR SMOKER ONLY):

        1.   DURATION OF SMOKING: Q29 (INT2_39)

        2.   AGE STARTED SMOKING:
                CURRENT SMOKER: AGE AT EXAM - DURATION OF SMOKING
                EX-SMOKER: AGE STOPPED SMOKING (Q27) - DURATION OF SMOKING

        3.   DAILY SMOKING AMOUNT (Q28): INT2_38

        4.   TOTAL SMOKING AMOUNT (PER PACK YEAR):
                PPY= (DAILY SMOKING AMOUNT * DURATION OF SMOKING) / 20
                   = (INT2_38 * INT2_39) / 20

    C.   OTHER TYPE OF SMOKING: INTERVIEW II, Q30-Q32

0 (NO)	IF (INT2_40='2' AND INT2_41='2' AND INT2_42='2')
1 (YES)	IF (INT2_40='1' OR INT2_41='1' OR INT2_42='1')

D. PASSIVE SMOKING
    0 (NO)   IF INT2_33=0
    1 (YES)   IF INT2_33 > 0
    DAILY EXPOSURE TIME (IN HOURS): INT2_33.

E. PARENTAL SMOKING:
    0 (NONE)   (INT2_31=2 OR INT2_31=3) AND (INT2_32=2 OR INT2_32=3)
    1 (ONE)   INT2_31=1 OR INT2_32=1
    2 (BOTH)   INT2_31=1 AND INT2_32=1

2. EDUCATION: PERSONAL INTERVIEW FORM II, Q15 - INT2_4

A. CONTINUOUS: INT2_4 (YEARS)

B. CATEGORICAL:

    i. THREE CATEGORIES (EDUCAT1):
        1 (LESS THAN HIGH SCHOOL) $0 <= INT2\_4 < 12$
        2 (HIGH SCHOOL GRADUATE
          AND/OR SOME COLLEGE) $12 <= INT2\_4 < 16$
        3 (COLLEGE GRADUATE) $INT2\_4 >= 16$

    ii. FOUR CATEGORIES (EDUCAT2):
        1 (LESS THAN NINE YEARS) $0 <= INT2\_4 <= 9$
        2 (SOME HIGH SCHOOL) $10 <= INT2\_4 <= 12$
        3 (SOME COLLEGE) $13 <= INT2\_4 <= 16$
        4 (COLLEGE GRADUATE) $INT2\_4 >= 16$

3. TOTAL DEGREE OF INDIAN BLOOD: INTERVIEW II, Q16

A. CONTINUOUS: INDIAN = (INT2_5 / INT2_6) * 100%

B. CATEGORICAL:
    0 (LESS THAN 25%) $0 < INDIAN < 25\%$
    1 (LESS THAN 50%) $25 <= INDIAN < 50\%$
    2 (50-74.9%) $50 <= INDIAN < 75\%$
    3 (75-99.9%) $75 <= INDIAN < 100\%$
    4 (FULL BLOODED) $INDIAN = 100\%$

4. INDIAN TRADITION: INTERVIEW II, Q35-Q38

A. SPEAK NATIVE LANGUAGE, INDYLANG
    0 (NO)   INT2_45='3' OR INT2_46='5'

<pre style="white-space: pre-wrap">   1 (YES)   INT2_45='1' OR '2' AND (INT2_46='1' OR '2' OR '3' OR '4')
</pre>

    B. USE TRADITIONAL MEDICINE/HERBS, INDYMED
        0 (NO)   INT2_47='5' OR '9'
        1 (YES)   INT2_47='1' OR '2' OR '3' OR '4'

    C. TRADITIONAL CEREMONIES, INDYCERE
        0 (NO)   INT2_48='5' OR '9'
        1 (YES)   INT2_48='1' OR '2' OR '3' OR '4'

5. STRESS: INTERVIEW II, Q42-Q46

    A. SLEEP LOSS, Q42, SLEPLOSS
        0 (NO)   INT2_52='1'
        1 (YES)   INT2_52='2' OR '3'

    B. STRAIN OR STRESS, Q43, STRAIN
        0 (NO)   INT2_53='1'
        1 (YES)   INT2_53='2' OR '3'

    C. OPEN ARGUMENTS, Q44, QUARREL
        0 (NO)   INT2_54='1' OR '2'
        1 (YES)   INT2_54='3' OR '4' OR '5'

    D. ALCOHOL PROBLEM OF HOUSEHOLD, Q45, HOUSETOH
        0 (NO)   INT2_53='1'
        1 (YES)   INT2_53='2'

    E. SIZE OF HOUSEHOLD, Q46, HOUSSIZE
        1 (SMALL) $INT2\_54 \leq 4$
        2 (MEDIAN) $4 < INT2\_54 < 10$
        3 (LARGE) $INT2\_54 \geq 10$

6. ALCOHOL USE

    A. ALCOHOL DRINKING STATUS, ETOHUSE, Q47-Q48
        0 (NEVER)   INT2_57='2'
        1 (EX-DRINKER) INT2_57='1' AND $(INT2\_59 \geq 12$ OR $INT2\_60 \geq 1)$
        2 (CURRENT)   INT2_57='1' AND $INT2\_60 = 0$

    B. BINGE DRINK
        1. DURING THE PAST MONTH, Q52
            0 (NO) $0 \leq INT2\_64 < 5$

1 (YES) $INT2\_64 \geq 5$

2. DURING THE PAST YEAR, Q53
   0 (NO) $0 \leq INT2\_65 < 5$
   1 (YES) $INT2\_65 \geq 5$

C. AMOUNT OF ALCOHOL INTAKE

7. SOCIOECONOMIC STATUS (SES)

   A. RECEIVING FEDERAL ASSISTANCE:
      1. FOOD STAMPS / WIC, Q56
         0 (NO) $INT2\_68 = 0$
         1 (YES) $INT2\_68 > 0$

      2. COMMODITY FOOD, Q57
         0 (NO) $INT2\_69 = 0$
         1 (YES) $INT2\_69 > 0$

      3. FEDERAL ASSISTANCE, FEDHELP
         0 (NO) $INT2\_68 = 0$ AND $INT2\_69 = 0$
         1 (YES) $INT2\_68 > 0$ OR $INT2\_69 > 0$

   B. SES (EDUCATION, FAMILY INCOME, ...)

      1. HOUSEHOLD INCOME, Q58: USE THE CATEGORIES LISTED IN THE QUESTIONNAIRE.

8. FAMILY HISTORY OF DISEASES - PERSONAL INTERVIEW II, FAMILY HISTORY

   A. CLASSIFICATION:
      1. PARENTAL, FOR RELATIONSHIP CODE 1 AND 2 (FH1 AND FH14)
      2. FIRST DEGREE FULL-BLOOD RELATIVES:
         RELATIONSHIP CODE: 1 (MOTHER), 2 (FATHER), 3 (SISTER), 5 (BROTHER), 7 (DAUGHTER), AND 8 (SON).
      3. ALL FIRST DEGREE RELATIVES, ALL CODES.

   B. DISEASE HISTORY
      1. HEART DISEASE: MI AND HD
      2. CARDIOVASCULAR DISEASE: MI, HD, HBP, CVA
      3. DIABETES: DM
      4. KIDNEY FAILURE: KF
      5. ARTHRITIS: AT

6. CANCER

9. MEDICAL HISTORY, MEDICAL HISTORY FORM

   A. PRESCRIBED MEDICATIONS: USE CATEGORIES IN THE MANUAL (p. 282)

1-	ANTIHISTAMINE (400)	2-	ANTIBIOTICS (812)
3-	ANTINEOPLASTIC RX (1000)	4-	BETA-BLOCKERS (1216)
5-	ANTICOAGULANTS (2000)	6-	CARDIAC DRUGS (2404)
7-	HYPOLIPIDEMIC (2406)	8-	HYPOTENSIVE (2408)
9-	ANALEGISTIC (2808)	10-	ASPIRIN (280892)
11-	ANTICONVULSANTS (2812)	12-	PSYCHOTHERAPY (2816)
13-	ADRENALS (6804)	14-	ORAL CONTRACEPTIVE (6812)
15-	DIURETICS (4028)	16-	GI DRUGS (5600)
17-	MENOPAUSAL ESTROGEN (6816)	18-	INSULIN (682008)
19-	SULFONYUREAS (682020)	20-	THYROID AGENTS (6836)
21-	OINTMENTS (8400)	22-	VITAMINS (8800)
23-	UNCLASSIFIED (9200)		

   B. HISTORY OF:
      1. GALLSTONE, Q3c
         0 (NO)   MED22='2'
         1 (YES)   MED22='1'

      2. ARTHRITIS, Q3d
         0 (NO)   MED23='2'
         1 (YES)   MED23='1'

      3. CANCER, Q3e
         0 (NO)   MED24='2'
         1 (YES)   MED24='1'

      4. KIDNEY FAILURE, Q3g
         0 (NO)   MED28='2'
         1 (YES)   MED28='1'

      5. EMPHYSEMA, Q3h
         0 (NO)   MED31='2'
         1 (YES)   MED31='1'

      6. LIVER CIRRHOSIS, Q3i
         0 (NO)   MED32='2'

| | 1 (YES) | MED32='1' |

7.   RENAL DIALYSIS, Q4a
      0 (NO)   MED42='2'
      1 (YES)   MED42='1'

8.   KIDNEY TRANSPLANT, Q4b
      0 (NO)   MED43='2'
      1 (YES)   MED43='1

10.   REPRODUCTION AND HORMONE USE (FEMALE ONLY), MEDICAL HISTORY

   A.   REPRODUCTION:
         1.   TIMES PREGNANT, Q7-1, REPRO1

         2.   NUMBER OF LIVE BIRTH, Q7-2, REPRO2

         3.   NUMBER OF LOST PREGNANCIES, Q7-3, REPRO3

         4.   NUMBER OF LIVING CHILDREN, Q7-4, REPRO4

         5.   MENOPAUSAL, Q8
               0 (NO)   REPRO5='2'
               1 (YES)   REPRO5='1'

         6.   AGE AT MENOPAUSE, Q9, REPRO6

   B.   HORMONE USE
         1.   ORAL CONTRACEPTIVE, Q11
               0 (NO)   REPRO9='2'
               1 (YES)   REPRO9='1'

         2.   AGE STARTED TO USE OC PILLS, Q12, REPRO10

         3.   TOTAL DURATION OF USING OC PILLS, Q13, REPRO11

         4.   EVER USE OF ESTROGEN OTHER THAN OC PILLS, Q14
               0 (NO)   REPRO12='2'
               1 (YES)   REPRO12='1' OR MEDICATION CODE (Q1a-Q1h)
                               CONTAINS '6816' (POST MENOPAUSAL ESTROGEN)

         5.   AGE STARTED TO USE ESTROGEN, Q15, REPRO13

6. TOTAL DURATION OF USING ESTROGEN, Q16, REPRO14

11. LAB DATA

    A. LIPID - CONTINUOUS VARIABLE
        1. TOTAL TRIGLYCERIDE, **ln(TRIG)**
        2. TOTAL CHOLESTEROL, CHOLEST
        3. HDL CHOLESTEROL, HDL_CHOL
        4. LDL CHOLESTEROL, LDL_CHOL
        5. VLDL TRIGLYCERIDE, VTRIG
        6. VLDL CHOLESTEROL, VCHOL
        7. RATIOS:
            i. VCHOL/VTRIG
            ii. HDL_CHOL/CHOLEST
            iii. HDL_CHOL/LDL_CHOL
            iv. APOB/(CHOLEST-HDL_CHOL)
            v. APOA1/HDL_CHOL
            vi. APOB/LDL_CHOL

    B. APOLIPOPROTEINS: APOA1, APOB

    C. GLUCOSE:
        1. FASTING BLOOD GLUCOSE, GLUC_0
        2. 2-HR BLOOD GLUCOSE, GLUC_2

    D. FIBRINOGEN

    E. PLASMA INSULIN

    F. FIBRINOGEN

    G. APO E PHENOTYPE

    H. PLASMA CREATININE

    I. URINARY ALBUMIN AND CREATININE

    J. GLYCATED LDL

    K. LP(a)

    L. LDL SIZE

M.   LDL TYPE

CUT POINTS FOR CONTINUOUS VARIABLES:

VARIABLES	LOW (0) NO	MEDIUM (1)	HIGH (2) YES

| AGEGP | 45-54 | 55-64 | 65-74 |

OBESITY: USING NHANES-II CRITERIA   *New= BMI ≥ 25*

OBESE	FEMALE:	BMI ≥ 32.3	
(95%)	MALE:	BMI ≥ ~~31.3~~ *31.1*	
OVERWT	FEMALE:	32.3 > BMI ≥ ~~27.8~~ *27.3 27.3* *BMI ≥ 30*	
(85%)	MALE:	31.1 > BMI ≥ ~~27.3~~ *27.8 27.8*	

| OBES_FAT | FEMALE:
MALE: | PCTFAT < 41%
PCTFAT < 29% | PCTFAT ≥ 41%
PCTFAT ≥ 29% |

| OBES_WHR
(65%) | FEMALE:
MALE: | WHR ≤ 0.98
WHR ≤ 0.96 | WHR > 0.98
WHR > 0.96 |

| TOTAL CHOLESTEROL
(NCEP GUIDELINE) | CHOLEST
< 200(mg/dl) | CHOLEST 200-239 | CHOLEST ≥ 240 |

| TOTAL TRIGLYCERIDES | TRIG < 250 (mg/dl) | | TRIG ≥ 250 |

| HDL CHOLESTEROL
(NCEP GUIDELINE) | HDL_CHOL < 35
(mg/dl) | | HDL_CHOL ≥ 35 |

| LDL CHOLESTEROL
(NCEP GUIDELINE) | LDL_CHOL
< 130(mg/dl) | LDL_CHOL 130-159 | LDL_CHOL ≥ 160 |

1.3   RECRUITING

In order to facilitate publicity and recruiting efforts in the community, it has been determined that individuals who participated in the Phase II exam are eligible for the clinical examination.

Eligible study participants are identified through the tribal population lists. Individuals will be contacted in an order convenient for each center. Local publicity campaigns and mailed information will alert participants before participation is requested.

When contacting an eligible examinee, the interviewer introduces the Strong Heart Study and explains its purpose and importance. A brochure and a letter explaining the purpose of the study and exam are used for recruitment. The voluntary nature of the study and the confidentiality of the collected data are stressed. If the subject is not at home at the time of the phone call or visit, call backs are made as necessary to meet the individual and schedule the clinic appointment. 100% participation is the goal.

In all areas, the recruiter should wear an identification badge. When scheduling appointments the recruiter should emphasize the following:

1.   That the volunteer should not eat breakfast the morning of the visit to the exam; and should not eat or drink anything but water after 9:00 p.m. the previous evening.

2.   That the volunteer should bring with him/her all medications which he/she has been prescribed and is currently taking;

3.   That the volunteer should not take their morning diabetes medication until blood drawing is completed;

4.   No tobacco or vigorous activity before the clinic visit;

5.   Volunteer should be instructed to wear loose clothing and ladies to wear a skirt and blouse or pants and shirt, rather than a dress

In case the volunteer might be retarded or otherwise mentally incapacitated, a surrogate must accompany him/her to the examination, preferably someone who is very familiar with the medical and family history.

The recruiter schedules the appointment with the clinic for each subject. Whenever possible, eligible members of a single household are scheduled on the same day. The recruiter should also verify name, address, and social security number at the time of the recruiting visit. When possible, participants should be reminded by phone or in person the day prior to the visit.

After the visit appointment is made, the clinic staff should assemble all forms and labels necessary for the exam and arrange to have the hospital chart for that participant available the morning of the clinic visit. The chart may also be reviewed to see if the participant satisfies the exclusion criteria for the glucose tolerance test.

1.4   PERSONAL INTERVIEW

1.4.1   Components of the Personal Interview

The personal interview is designed to obtain demographic information, medical history, health behavior, acculturation and stress data that are considered important in identifying risk factors for cardiovascular disease. A total of five questionnaires will be administered during the clinical examination:

1.   Personal Interview Form (I and II)
2.   Medical History Form
3.   Dietary Form
4.   Psychosocial Form
5.   Quality of Life

Personal living habits such as dietary, cigarette smoking and alcohol consumption, and stress have been considered as important risk factors for cardiovascular disease. Data on these factors as well as demographic information and the degree of acculturation will be collected by using the Personal Interview Forms (I and II) given in Appendix 3. Appendix 4 gives the Medical History Form which consists of questions on medical conditions, medications used and the Rose Questionnaire for angina pectoris and intermittent claudication.

Note: The Personal Interview Form I contains personal identification information. For confidentiality purposes, it should be sent to the Coordinating Center separately.

1.4.2   Guidelines for Interviewers

1.   Introduction

The personal interview is probably one of the most important procedure for data collection in epidemiologic research. The personal interview has been shown to increase response over self-administered questionnaires, and when rapport is established between the interviewer and the interviewee it has been shown to be an excellent source of high quality information for epidemiologic research purposes. However, the interviewer must be able to show tact, care, and sensitivity, to be effective. Not everyone can become a successful interviewer.

Also, the personal interview can lead to a lack of standardization in the data collected, particularly in a multicenter study such as the Strong Heart Study. Since the interviewer is known to have a large effect on the quality of the data obtained, therefore interviewer training is very important. Please read this interviewer's manual frequently, and refer to it as needed during the study. It is also recommended that each Study Coordinator, hold monthly interviewer meetings, to go over common problems and clear up any questions about the interview procedures and the interview forms in the Strong Heart Study.

2.   Types of Interviews

Structured versus Unstructured Interviews

In an unstructured interview the responses to questions are open-ended, and information given is to be recorded as given. In a structured interview the questions are usually closed, with a specific set of answers provided in the questionnaire.

For the Strong Heart Study we are using both structured and unstructured interviews. The use of structured interviews is the best way to maintain consistency in the data being collected. Interviewer training is important in order to maintain as much consistency in the interviews between study centers as possible.

Because we are using structured and unstructured interviews, we can achieve even more consistency if all interviewers conduct the interviews in a similar way. Therefore, ask each question as it is written. Do not reword the question. Also, ask the questions in the order they are given in the interview form. Hopefully, by following these procedures we can achieve a high degree of consistency in the way the interviews are conducted.

3.   Style of the Interview

The interview style is also important and some of the components of what is generally considered to be acceptable interview style are listed below. In addition to the components of style listed below, the following interviewer characteristics are also very important: Politeness is very important since we will be asking sensitive questions to strangers, in a situation where they may be uncomfortable. Sensitivity on the part of the interviewer is also important, in order to know how and when to be more or less assertive in asking for information. Besides these qualities, listed below are what we expect our interviewer's style to approach, so please try to develop your style in accordance with these guidelines:

a. Non-judgmental, non-evaluative style. A large portion of the impression which the respondent has of the interviewer is based solely on the interviewer's voice and the manner with which the interviewer responds to the respondent's comments. A judgmental or evaluative response would indicate that the interviewer has made a judgement of the relative goodness, appropriateness, effectiveness, or rightness of the respondent's statement. The interviewer should not, in response to the respondent's statements, state what the respondent should or should not do in a given situation. The interviewers task is simply to record the information provided, and to elicit the desired response.

b. Non-interpretive style. As above, the interviewer should not use a style that might be considered teaching or preaching. An interpretive response is one which indicates that the interviewer's intent is to teach. We are interested in the respondent's impression of what was happening, not in the interviewer's impression.

c. Allow for respondent to complete sentences. Do not try to help the respondent by answering the questions for him/her. No matter how slowly the respondent is speaking, putting words in the respondent's mouth or not allowing the respondent to finish thoughts will generally alter the information which the respondent is attempting to give. However, long hesitations may be bridged by asking appropriate questions.

d. Supportive remarks. Remarks which indicate that the intent of the interviewer is to reassure, to pacify, or to reduce the intensity of the respondent's feelings are appropriate. However, these should be in keeping with local terms and expressions, and should be short so as not to detract from the interview itself.

e. Probing. This is an important response style which will be discussed further. A probe is a response which indicates that the interviewer's intent is to seek further information, to provoke further discussion along a certain line, or to question the respondent. Direct probes will be specific questions about details of what the respondent said.

f. Non-directive, or understanding. A typical non-directive response might be "I see". This is the general idea of understanding murmuring or clucking. The interviewer might also repeat what the respondent just said. This may prompt the respondent to elaborate.

4. Gain Rapport with the Interviewee before Commencing Interview

The first step in gaining the confidence of the respondent is a straightforward, believable introduction of the interview and the reason for this contact. It may help in gaining rapport with the respondent if you tell him/her a little about yourself, such as where you are from, and your background, etc. If the respondent seems to hesitate or has some questions the interviewer must be prepared with a more detailed explanation of why the information is needed. Also, if the respondent raises the issue of the confidentiality of the information collected, the interviewer must be prepared to reassure him/her of the precautions taken to respect their privacy.

5.   Interviewer Error

We should try to minimize interviewer error during this study. The primary objectives of epidemiologic research are (1) to obtain measurements of exposure and disease variables relevant to the objectives of the study, and (2) to minimize error in these measurements. The presence of an interviewer may both reduce error and increase error. It may reduce error by increasing the response rate, motivating the subject to respond well and probing to obtain complete data when the responses volunteered fall short of what is desired. The presence of an interviewer may increase error if by his or her appearance, manner, method of administration of the questionnaire or method of recording of the responses he or she exerts a qualitative influence on the subject's responses. Possible sources of error in the interview for data collection include (1) conditions of administration (heat, light, ventilation, freedom from distraction, lack of time, etc.); (2) interaction of the personality (sex or race of the interviewer with that of the subject); and, (3) performance by the interviewer (questioning, prompting and recording of responses).

The following are the common interviewer errors :

a.   Asking errors. Omitting questions or changing the wording of questions.

b.   Probing errors. Failing to probe when necessary, biased probing, irrelevant probing, inadequate probing, preventing the respondent from saying all he or she wishes to say.

c.   Recording errors. Recording something not said, not recording something said, incorrectly recording response.

d.   Flagrant cheating. Not asking a question but recording a response, recording a response when the respondent does not answer the question asked. These kinds of errors do occur and this has been amply documented by various studies. Cheating has been shown to be more common when the

interviewer is in an uncomfortable situation with the interviewee, i.e., he/she is difficult. We should try to be aware of these situations to avoid the interviewer errors above.

6. Circumstances for the Interview

We will not have very much control over the circumstances for the interviews. However the following should be considered in arranging for conducting interviews:

a. Time. There will be little control over the time of the interviews, since we will have many different interviews to carry out over a short period of time. However, it is desirable not to interview until after the breakfast has been served, otherwise the interviewee may tend to be somewhat uncomfortable.

b. Place. The place for the interview should be chosen where there are as few distractions as possible. Try to select a place where the location is quiet and comfortable. If it is possible, it is ideal to sit at a table, with the interviewer facing the interviewee, so that the interviewer can organize the papers. Privacy is also important. If the respondent will need to refer to records during the interview, be sure that the records are available before the interview begins.

7. Asking Procedures

In general the rules for asking questions in structured interviews can be summarized as follows:

a. Questions must be asked according to the instructions for each form. Be sure to read and re-read the instructions for each questionnaire you are using, and to ask all the questions in the same way to each person interviewed.

b. Read the questions exactly as they are worded in the questionnaire. If the question is misunderstood, then it may be repeated, interchanging local terms, if necessary for understanding.

c. Read each question slowly.

d. Use correct intonation and emphasis.

e. Ask the questions in the order that they are presented in the questionnaire.

f.   Ask every question that applies to the respondent (all inapplicable questions will be identified as such by skip instructions in the questionnaire).

g.   Repeat questions IN FULL that are misheard or misunderstood.

h.   Read all linking or transitional statements exactly as they are printed.

i.   Do not add apologies or explanations for questions unless they are printed in the questionnaire.

PROBING: Probes are additional questions asked or statements made by the interviewer when the answer given by a respondent is incomplete or irrelevant. Probing has two major functions : (1) To motivate the respondent to reply more fully; (2) to help the respondent focus on the specific content of the question. It must fulfill these functions without biasing the respondent's answers. However, probes, when they are used, MUST be neutral. Probing can introduce bias, such as by summarizing your understanding of the response to the subject when an unclear response has been given, or by offering some alternative interpretations from which the respondent can choose, and this must be guarded against.

The following are NON-DIRECTIVE methods of probing:

a.   Repeat the question (RQ). All that may be required to clear up a vague answer may be to repeat the question. You may begin by saying "I am not sure that I understood you, let me just repeat the question so that I can be sure to get your answer right."

b.   The expectant pause. Waiting expectantly will tell the respondent that the interviewer is expecting more information than has been provided.

c.   Repeat the reply. Repeating the reply aloud while recording it may stimulate the respondent to provide more details.

d.   Neutral questions or comments. Various neutral probes may be used for purposes such as clarification, specificity, or completeness: "What do you mean exactly?", "In what way?", "Could you be more specific about that?", "Anything else?", "Can you tell me more about it?"

e.   Interpretation. Make sure that the question was understood, since that may be the reason for incomplete answers. You may need to ask the question again, perhaps substituting some local terms, If there is a problem in interpretation.

FEEDBACK: The provision of feedback by the interviewer to the respondent about his or her performance has been the subject of much research. Some studies have shown that the use of feedback in health-related surveys increased the amount of reporting of most events. Your decision about whether to provide feedback may depend upon

the performance of the person you are interviewing, and your experience in the benefits of providing feedback.

8.   Specific Instructions for Telephone Interviewing

While it is generally held that the principles outlined above, which have been derived solely from research into and experience of face-to-face interviewing, apply to telephone interviewing, the evidence that this is true is very limited. Telephone interviewing is probably not simply the transfer of face-to-face techniques to the telephone. Use of visual cues, such as "show cards", is impossible on the telephone and must be compensated for in questionnaire design. There is evidence that this compensation may lead to response differences. In addition, other non-verbal communication, both from the interviewer to respondent and respondent to interviewer, is absent. The "expectant pause", for example, may be much more difficult to use as a probe for additional information on the telephone. It is also more difficult for the interviewer to establish the legitimacy of the interview on the telephone and the pace of the interview may be faster (because of the need to keep talking) leading to hurried and, perhaps, less thoughtful responses. On the positive side, the telephone should eliminate non-verbal biasing activity by the interviewer, and may encourage more honest reporting of threatening behaviors. Empirical data, however, have not shown consistent evidence of these effects.

9.   Instructions for Recording Responses

In the study manual, each interview and form contains a set of question by question instructions for filling out the interview form, to clearly describe the information that is being solicited. These instructions should be read carefully and understood before attempting to fill out an interview form.

In addition, see the attached instructions for filling out forms. The following are some additional guidelines for recording responses:

a.   Make sure that you understand each response.

b.   Make sure that the response is adequate.

c.   Do not answer for the respondent (i.e., do not infer a response from an incomplete or inadequate reply).

d.   Begin writing as soon as the respondent begins talking. (The respondent's interest may be held by repeating the response aloud as you are writing).

e.   Use the respondent's own words and record the answers verbatim.

f.   Include everything that pertains to the question's objectives.

g.   Note in the questionnaire the nature and place of each probe used.

h.   Do not erase anything. If a response is wrong, strike it out and enter the correct response.

i.   Write "refused" beside any question that the respondent refused to answer.

1.4.3   Training & Quality Control of Interviewers

1.   Training

Interviewers will be centrally trained in July, 1993 at the training session in South Dakota using a standardized procedure for administering each questionnaire. Training will include instructions in research interviewing techniques and in completing each form. Interviewer skill training will include:

(a)   adherence to the standardized protocol
(b)   use of non-judgmental attitudes
(c)   degree and nature of prompting permitted
(d)   dealing with problem interviewing situations
(e)   handling participants comments and recording relevant information on the note logs
(f)   post interview responsibility for the data

2.   Quality control of interviewers

To insure consistency and accuracy and to minimize inter- and intra-interviewer differences, study coordinators will monitor 5% of the interviews done by each interviewer.

1.5   RATIONALE FOR MEASUREMENTS

1.5.1   Blood Pressure

As blood pressure rises, so does risk of ischemic heart disease and its complications. The range of normal blood pressures is wide. Even within the "normal range", risk increases as the upper limits are approached. Usually, blood pressures are expressed as systolic pressure/diastolic pressure; values 140/90 mmHg or higher are considered to be hypertensive for adults. Middle-aged persons with a diastolic blood pressure of 90-104 mmHg (so called "mild" hypertension) have a risk of heart attack that is about 70 percent higher than that of persons with a diastolic pressure under 80 mmHg (normal value). Persons with a diastolic blood pressure exceeding 104 mmHG (moderately severe to severe hypertension) have a risk more than twice that of those with a normal value. Hypertension is an especially strong risk factor for stroke and, to a lesser extent, for peripheral vascular disease. Most of the knowledge of the consequences of high blood pressure arises from studies of sitting arm blood pressure.

1.5.2   Measurement of Body Fat

Population studies have always demonstrated a univariate association between obesity and CVD. However, in many early studies, the association between obesity and the incidence of CVD did not remain significant in multivariate analysis, and thus it was thought that obesity was a risk factor solely because of its influence on other risk factors such as blood pressure, plasma lipoproteins and diabetes. More recently, especially in longer term studies, significant independent associations between obesity and the incidence of CVD have been demonstrated.

Although early records are not conclusive, all evidence indicates that obesity among American Indians was rare until the last century. Their farming and hunting life styles which were associated with high degrees of physical activity and the lack of consistently abundant food sources, probably assured the maintenance of a lean population. However, with the advent of "Westernization" and the reservation system obesity has increased steadily among all Indian tribes and is now a major health problem. It is thus essential to evaluate the extent of obesity in the individuals in this study in order to ascertain its role in cardiovascular disease as well as its relationship to risk factors such as diabetes and hypertension.

In the past, assessment of obesity in population studies was invariably accomplished either by algorithms such as ratios of weight to height, or by measurements of skin folds using calipers. This was because assessment of body composition required either very expensive equipment or time consuming procedures, such as underwater weighing. Within the past few years, instrumentation has become available to allow estimates of body composition from measurements of tetrapolar impedance. This

measurement of bioelectrical impedance is quick and easy to perform and has been extensively validated against densitometry. These validations were first performed by Lukaski et. al. (53) and by Roche et. al. (54), in a wide variety of individuals. The conductivity increases in individuals with low percent body fat and the instrumentation calculates the percent body fat utilizing a computerized algorithm.

1.5.3 Anthropometric Measurements

It has been recently demonstrated that among obese individuals, the distribution of body fat is related to certain patterns of morbidity. Vague and co-workers (55) have observed that body fat distribution differs among obese individuals, and that obese subjects can be roughly divided into two groups depending on whether accumulation of body fat is subcutaneous and peripheral (referred to as gynecoid or female type obesity) or whether the fat accumulation is central and primarily in the omentum (referred to as central or android obesity). The latter distribution has been shown in a number of studies to be consistently associated with the triad of hypertension, insulin resistance, and cardiovascular disease. Most studies have shown that central obesity is a risk factor for coronary artery disease.

No systematic studies of body fat distribution have been made among the American Indians. However, visual observations suggest that central obesity is much more prevalent among this racial group.

The quantification of central vs. peripheral obesity is not well standardized. Original studies were done simply by photographs and visual evaluations. This was supplanted by body circumference measurements with investigators generally taking the ratio of the body circumference at the waist to the hip or the thigh as a measure of fat distribution. However, it is clear that the body fat of interest in central obesity is the non-subcutaneous, and therefore, whole body scanning devices are necessary for a precise evaluation of this depot. Nevertheless, it has been shown in a number of population studies that the comparative circumference measurements are an approximation of the body fat distribution and the only practical techniques usable in a field study.

1.5.4 Measurements of Peripheral Vascular Disease

The atherosclerotic process affects vessels in many parts of the body. While the most conspicuous morbidity and mortality arise from coronary atherosclerosis, large vessel peripheral arterial disease (PAD) often results in significant incapacitation of the lower extremities and has been also strongly associated with the incidence of coronary heart disease. Criqui and co-workers have shown that large vessel PAD is strongly and significantly predictive of all caused mortality in both sexes with a relative risk of 4 to 5, and this was independent of other cardiovascular risk factors in a multivariate analysis. Moreover, data from the Framingham study indicate that diabetes was associated with an

even greater magnitude of increase of peripheral vascular disease than was coronary heart disease.

A thorough evaluation of peripheral arterial occlusive disease usually entails both a history and a physical examination including measurements of pulses and segmental blood pressures and then more complex measures such as angiography or sonography. The latter two techniques are both expensive and difficult to apply in a field setting. On the other hand, both Criqui et al (56) and Beach et al (57) have used segmental blood pressures measured by a simple doppler instrument in studies involving hundreds of patients. In addition, the correlation between quantitative velocity measurements and segmental blood pressures with occlusion as measured directly by angiography has been established.

Because of time limitations and economic consideration for purchase of equipment, the following indices of peripheral vascular disease will be made in this study.

1. Rose Questionnaire for intermittent claudication.
2. Palpation of posterior tibial and dorsalis pedis pulses.
3. Auscultation for femoral bruits.
4. Measurement of the ratio between blood pressures taken at the antecubital fossa (brachial) and ankle (posterior tibial) using a doppler listening device (Imex Mascot Model).

1.5.5   Examination of the Lungs, Carotids and Neck Veins

The questionnaire currently used by Kriska, et al. (58) from the University of Pittsburgh, on the Gila River Reservation will be modified and adapted for this study. This consists of an interviewer administered questionnaire to assess general, leisure and occupational activities. It is designed primarily to evaluate the past year and past week activities and a summary measure of lifetime physical activity.

1. The lungs

Auscultation is done to detect signs of congestive heart failure (rales).

2. Carotids

Carotid bruits could be due to vascular disease or aortic valvular disease.

3. Neck veins

Dilatation in the upright position usually indicates congestive heart failure.

1.5.6 Electrocardiograms

All participants will have a resting electrocardiogram so that evidence for ischemic changes and left ventricular hypertrophy can be determined. The prevalence of such changes will reflect the prevalence in the population studied and can be compared to other population-based studies and among the three sites.

1.5.7. Overview of Laboratory Measurements

1.   Lipoprotein Profile

Lipoprotein Physiology: Lipoproteins are basically spherical particles ranging widely in size and composed of two components: the lipids (or fats) in the core of the particle and the proteins on the surface of the particle. The two types of lipids which we are interested in measuring as part of the present research are triglyceride (TG) and cholesterol (CHOL). Depending on the relative amount of these two components and various associated proteins, different classes of lipoproteins can be defined (Table 1.1).

Table 1.1: Definition of Lipoproteins

Class	%Lipid	% Protein	Origin and Function
Chylomicrons	99	1	Intestine; transport of newly absorbed dietary fats; normally not detectable in plasma after a 12-hr fast; creamy layer on top of plasma tube after 12 hrs in the refrigerator.
VLDL, very low density lipoprotein	90	10	Liver; transport of newly synthesized triglycerides to peripheral tissue; approximately 80% of plasma TG is in this fraction
LDL, high low density lipoproteins	75	25	Liver; derived from VLDL after the triglycerides have been metabolized; transport of cholesterol; approximately 75% of plasma cholesterol is in this fraction
HDL, high density lipoproteins	45	55	Liver and intestine transport of cholesterol from peripheral tissues back to the liver

The evidence is overwhelming from both cross-sectional and prospective studies in a wide variety of populations that total and LDL cholesterol are significantly associated with the occurrence of atherosclerotic coronary vascular disease (ASCVD), and the HDL cholesterol has a negative or "protective" effect.

The relationship with total triglycerides or VLDL triglycerides has been more controversial. Several population studies have now demonstrated an independent positive association between elevated triglycerides and ASCVD. Triglycerides are also closely linked to obesity, hyperglycemia and low HDL, and are therefore important to measure because of their reflection of these disorders. Some of the ambiguity concerning the associations between triglycerides and coronary vascular disease stems from the possibility that all elevations in triglycerides may not be equal. That is, elevated VLDL with a high proportion of protein, or cholesterol rich VLDL such as that observed in many diabetics may be more atherogenic than large, triglyceride-rich VLDL.

If a Beta Estimate is performed, measurements are made of total plasma cholesterol and triglyceride. HDL is measured after precipitation of LDL and VLDL. LDL is calculated by the Friedewald formula:

LDL chol = Total Chol - HDL Chol - (Total TG/5)

This estimate is based on the assumptions that VLDL cholesterol is a minor portion of the total cholesterol, that the majority of the total triglyceride is in VLDL, and that the composition of the VLDL is "normal", that is, VLDL cholesterol is approximately one fifth that of triglyceride.

This method has two advantages:

(1)   Can be performed on plasma that was frozen.
(2)   Requires much less technician time.

The disadvantages are:

(1)   It is inaccurate in individuals with high triglycerides (> 400).
(2)   It is inaccurate in individuals with altered VLDL composition.
(3)   It will not allow the isolation and examination of VLDL composition and relations to ASCVD.

If Beta quantitation is performed, total cholesterol and triglyceride are measured, and HDL cholesterol is measured after precipitation, as in the beta estimate. In addition, VLDL is isolated by ultracentrifugation, and the ratio of

cholesterol to triglyceride is measured in VLDL. From this we can directly calculate:

LDL-Chol= Bottom-chol - HDL-CHOL
VLDL-Chol= Total chol - Bottom chol
VLDL-Triglyceride = VLDL-Chol x [VLDL-TG/VLDL-Chol]

The advantages are:

(1)   LDL cholesterol is measured directly, not estimated.
(2)   A measure of VLDL composition is obtained.
(3)   VLDL and bottom fractions are available for further apoprotein measurements or for storage.

The disadvantages are:

(1)   The ultracentrifugation is laborious, time consuming and costly.

The Beta quantitation procedure is selected because of the need for accuracy in the measurement of LDL and because it yields a VLDL fraction of particular interest in a population with high prevalence of diabetes. People with diabetes frequently have abnormal composition of VLDL.

2.   Glucose Tolerance Test (Glucose and Insulin)

Although it may be argued that 75 gm glucose load is not a measure of glucose disposal that is analogous to carbohydrate ingested during daily meals, it is the standard measure of glucose tolerance which can be compared to other studies, and forms the basis for all the currently used criteria for diagnosis of diabetes. Because of the high prevalence of diabetes in all three centers, and because of the multiple previous studies reporting associations between diabetes and CVD, a glucose tolerance test is essential for the current study. The most simple to perform is one where blood samples are drawn by venipuncture at fasting, and then two hours after ingestion of the glucose. All other fasting blood samples may be obtained at the time of the fasting sample, thus limiting the venipunctures to two.

Glucose concentrations will be measured in both fasting and two hour samples. Blood for this is obtained in tubes containing fluoride to prevent consumption of glucose by WBCs. Previous studies in Phoenix have shown that tubes of blood containing fluoride can be held on ice for up four hours before isolating the plasma, and glucose values are stable. Glucose is measured on the Hitachi analyzer using a glucose oxidase technique.

Insulin concentration in blood has been reported in several recent studies to be an independent risk factor for the development of CVD. Although the mechanism of this association has not been established, there are several intriguing possibilities involving its link with insulin resistance, hypertension, hypertriglyceridemia, and thrombosis. The first three factors have been linked in several population studies in individuals with central obesity. However, some studies suggest that these factors are not universally associated. It will thus be of interest to measure fasting insulin concentrations in individuals at the three centers, to evaluate its relationship to vascular disease and also to blood pressure, triglycerides, waist/hip ratio and fibrinogen.

Insulin will be measured using an overnight radioimmunoassay developed as a modification of the method of Morgan and Lazarow (50). It utilizes a double antibody method; both antibodies and labeled insulin can be obtained efficiently from commercial sources. Although no absolute reference plasma pools are available for insulin, we have constructed our own control pools. The assay has proven to be stable over time with a coefficient of variation of 8-10%. One source of error in insulin measurements occurs in some individuals who have been previously treated with insulin, and thus have circulating insulin antibodies. Samples from insulin treated diabetics will be flagged at the time of drawing, so that their data can be separately evaluated.

3.   Glycated Hemoglobin

The relationship between glycemia and the occurrence of CVD is an important one. Although it is well established that diabetes is associated with an increase in CVD, it is not clear whether there is a significant correlation between plasma glucose and either prevalence or incidence of CVD, and in fact several studies have failed to show a relationship between macrovascular disease and glucose tolerance, especially in diabetics.

One explanation that has been cited for this is that tests such as GTT do not reflect long term glycemia, and also have high intra-subject variability. An alternative for the integrated assessment of glucose levels over time is the measurement of glycated proteins, since the nonenzymatic glycation of proteins is a constant process which increases directly with increasing concentrations of glucose. Thus, the measure of extent of glycation of a protein with a relatively long half-life is an assessment of the ambient levels of glucose during the life of that protein.

The most commonly employed is the measure of Hemoglobin Alc. It can provide an assessment of glucose status which reflects approximately a two month period. Although there is an excellent correlation between HbAlc and glucose during GTT over the entire range of glucose intolerance, the correlation in the

nondiabetic to IGT range is not as strong. A recent measure of HBAlc in Framingham has shown a very strong positive correlation between it and CVD over the entire range.

HbAlc is much more laborious to measure than is glucose. Although several electrophoretic and chromatographic techniques have been employed in the past, currently an HPLC assay appears to be the method of choice. HbAlc will be measured by HPLC in the laboratory.

4.   Fibrinogen

Disorders of the coagulation system could play a major role in ASCVD. There has been special interest in the role that abnormalities in the clotting system might play in the increased risk for atherosclerosis observed in diabetics. Abnormalities in several factors have been reported to be associated with atherosclerosis. One of the most commonly and easily studied is fibrinogen, and it has been shown to be an independent risk factor for CVD in both nondiabetics and diabetics in the Framingham study.

Fibrinogen has been most commonly measured using a chronometric technique. For this thrombin is added to plasma to induce clotting, and the clot is quantitated on a fibrometer or automated coagulometer. Since the lab at the MRI does not possess this equipment, measurements will be made by Dr. Russell Tracy at the University of Vermont.

5.   DNA

Because CVD is a clinically heterogeneous disorder and involves a complex interaction between genetic and environmental factors, it will probably be explained by a complex polygenic transmission. Recent development in recombinant DNA technology, including using restriction enzymes to identify polymorphisms, are now frequently being used in study of genetic disorders and may be very helpful in sorting out the genetics of complex diseases such as atherosclerosis. Methods are now available for detecting altered nucleotide sequence in the human genome, which may be used as genetic markers of CVD or risk factors. Certain alterations in DNA sequence may be demonstrated by cleaving genomic DNA with restriction enzymes, hybridizing with cloned DNA probes and by detecting changes in the length of gene fragments by autoradiography. These techniques have allowed the chromosomal mapping of the genes for diseases such as muscular dystrophy and Huntington's chorea.

Although we do not yet have evidence in Indians that CVD shows familial aggregation, there is certainly ample indication in other populations that CVD and

several of its risk factors are familial and thus would lend themselves to genetic studies. Although genes for cardiovascular disease have been localized in animals, attention in human studies has been focused on identifying alleles that may be associated or linked with other diseases. Since diabetes, hypertension and altered lipoprotein concentrations are strong risk factors for the development of CVD, attention has been focused on the possibility that abnormalities in apoprotein or insulin gene loci might be associated with susceptibility to CVD. Mandrup-Poulsen et al. (60) have suggested that a polymorphic region of DNA close to the human insulin gene is a genetic marker for atherosclerosis. Karathanasis et al. (61) have shown that the genes for ApoA-I and apoC-III are physically linked, and that polymorphism of the apoA-I gene inherited as a trait linked to premature atherosclerosis in one affected family. Ordovas et al. (62) have also shown that the apolipoprotein A-I gene polymorphism was associated with CAD in a study of 88 patients, and was also found in 8 out of 12 kindreds with familial hypoalphalipoproteinemia. Finally, the possible association between NIDDM and arteriosclerosis is further suggested by a recent report of an association between a apoA-I gene polymorphism and susceptibility to NIDDM.

Because of the distinct possibility that the next several years will lead to greatly increased availability of genetic markers and likely specific gene loci with documented association with CVD, it is of interest to study these in the Indian groups to be examined in the current survey. The present study will undoubtedly include many related people and gives the opportunity to identify families for linkage studies. For this reason it is proposed in the present protocol to isolate and store DNA from lymphocytes of blood sample. This can be easily accomplished in an efficient and economical way and would, therefore, serve as a store for future genetic studies.

6.   Lipoprotein(a)

Lp(a) is a heterogenous lipoprotein class which consists of an LDL particle to which a large glycoprotein moiety - apo(a) - is attached via a disulfide bridge. It was first identified by Berg[66] as a genetic variation of the immunologic response in rabbits to human LDL. In 1972, interest in Lp(a) was heightened by 3 independent reports[67, 68, 69] suggesting a link between the presence of a pre-beta migrating lipoprotein fraction[66] identified shortly thereafter as Lp(a) and patients with coronary artery disease. Subsequent retrospective[66,67] as well as prospective[66] studies have confirmed this association. Elevated levels of Lp(a) have also been associated with the prevalence of cerebrovascular disease[67] and coronary artery bypass restenosis. Lp(a) shares structural homology with plasminogen activator inhibitor and it may thus interfere with fibrinolysis.[75] The impact of co-existent hyperglycemia or diabetes on Lp(a) concentrations and function is controversial.[76]

7.　　Genetic Admixture

The relative proportions of genes that parental populations contribute to a hybrid population can be estimated from allele frequency distributions when the parental populations have been surveyed for the genetic markers used in the analysis. The power of a particular genetic polymorphism to estimate admixture proportions depends upon the degree of variation at the genetic locus and the relative differences in allele frequencies between the parental groups. For instance, in Native Americans, when estimating the proportion of Caucasian admixture, the Gm allotype system is very powerful because it has five major haplotypes and one of these, Gm3:5,13,14, has a very high frequency in European Americans, 0.650, but is absent in non-admixed Native Americans. However, modern mathematical techniques do not rely on one allele or haplotype alone. Rather, they incorporate all of the alleles and haplotypes over multiple loci. The Gm and Km systems, and the traditional red blood cell loci, ABO, MNSs, Rhesus, Duffy, and Kidd, provide the starting point for the method of pooled admixture estimates that is within the budget and logistical constraints of the study. Together they represent 24 alleles and haplotypes that are well characterized in European, African, and Native Americans.

Pooled admixture analyses using these multiallelic techniques have led to a better understanding of genetic admixture in human populations and its effect on genetic epidemiology. Williams, et al,[77] have shown that pooled admixture estimates in the Pima and Tohono O'odom Native Americans of the Gila River Indian Community are highly correlated with estimates derived from stated admixture. This suggests that the subjects are generally aware of their amount of non-Indian alleles. Knowler, et al,[78] have shown in this same population that the genetic admixture can be a confounding variable in disease-allele association studies, and that it is important to have a control on the amount of non-Indian alleles in a Native American community that is the subject of an epidemiological study. The pooled admixture analyses can also be jointed with demographic data to present a complete description of the relative proportions of Native American and non-Indian alleles and to describe the populations from which the genetic admixture was derived[79].

Methods for individual admixture estimates, the proportion of admixture in an individual rather than a population, have been worked out by Chakraborty and co-workers[80, 81] and applied to studies of Mexican Americans in Texas. These techniques, and modifications that are being prepared by Robert C. Williams and Jeffrey Long, also use allele and haplotype frequencies that have been well characterized in the parental populations. However, because the reliability of the individual proportion improves as more polymorphic loci are added, in the future, consideration will be given to adding DNA systems, particularly the highly polymorphic VNTR markers and the short, tandem repeat loci.

Both pooled and individual estimates of genetic admixture will be calculated for the Strong Heart sample. First the Gm and Km systems will be employed to determine the amount of genetic admixture in the entire sample of Native Americans, and within subsets of this sample, by tribe. The magnitude of this admixture will then be analyzed with respect to geographic location to determine whether there is a cline for genetic admixture in Native Americans. When the red blood loci are finished, they will be added to the Gm and Km systems to refine the above estimates and to calculate the individual admixture values and weights for each subject in the sample. These pooled and individual admixture values can then be compared with the prevalence of cardiovascular disease in the Strong Heart sample to determine whether non-Indian alleles are a risk factor.

1.6   PROCEDURE FOR GLUCOSE TOLERANCE TEST (GTT)

For all subjects, a fasting glucose value will first be obtained by using One-Touch (see Section 6.1.1 for procedure). Query subjects as to whether they are a known diabetic. If they are, ask if they take insulin or oral agents.

Note that all diabetic participants taking insulin will be exempted from the glucose tolerance test (GTT). Those diabetics who take oral agents and who have two random glucose values ≥ 250 mg/dl. or any participant with a fasting glucose ≥ 225 mg/dl. by One-Touch will also be EXEMPTED from the GTT. For individuals on renal dialysis or who have had a kidney transplant, blood will be drawn at the time of the examination, if possible.

1.   Have the bottle of glucose (Glutol 75g.) and blood drawing equipment ready. Although the Glutol has proved to be very dependable and consistent when its concentration per ml has been measured in numerous samples, the volume supplied per bottle is not consistent. Thus it is necessary to measure both the Glutol and the water into which it will be diluted for each patient. The easiest way to accomplish this is to have a plastic graduated container such as that used for urine collections. The person administering the glucose tolerance test can thus pour 135 ml of Glutol into the measuring container, pour that into the cup supplied to the patient and then measure out 135 ml of water using the same measuring container. The cup containing the diluted Glutol is then ready for the patient. This measuring container can be used for all patients visiting on that day, but it should be discarded at the end of the day and a fresh measuring container used for the next clinic.

2.   Ask subject if he/she has been fasting for 12 hours and whether he/she has refrained from smoking and beverages other than water, and record the response on the GTT check list given in Appendix 6.

3.   Draw fasting blood samples as described in Procedure for Blood Drawing. Record the time of blood collection.

4.   Describe the purpose of the GTT to the subject.

5.   Ask the subject to drink the glucose solution quickly, within 3 minutes - Record the time the process started on form.

6.   Instruct the subject that he/she should not eat, drink or smoke anything until the second blood sample is obtained two hours later.

7.   Instruct the subject to take the specimen container to the bathroom for the urine sample - Record the time of urine collection.

8.   Place urine sample in the refrigerator.

9.   Obtain second blood sample at exactly 2 hours post load - Effort should be made so that the second blood sample is obtained at exactly 2 hrs $\pm$ 3 min. Record the time of collection.

1.7   PHYSICAL EXAMINATION

During the examination, participants wear a gown, or loose fitting clothes that do not impair accurate body measurements and the examination. It is helpful to have them wear large scrub pants to enable the pant legs to be rolled up for the ECG examinations. Shoes and socks are removed for the supine examination and weight and height measurements. The form to be used is given in Appendix 5.

1.7.1 Anthropometry

Anthropometry is performed before the clinic snack with the participant's bladder empty. The subject may wear a scrub suit or clothing into the station. Measurements may be taken over the scrubsuit or light clothing only. Make sure that the pockets are empty and the belt is removed. Height and weight measurements are not to be taken with the participant wearing shoes.

Measurements if possible are taken by a team of two persons (one acting as observer, the other as recorder). If two are available, the first observer takes the measurements, calling out the value of the measurement.

The first observer keeps the measuring instrument in place until the recorder repeats the number. The recorder also checks the examinee's position during the procedure. If a single observer performs the measurements, each should be recorded immediately after they are taken. Values taken are rounded to the nearest unit indicated for each measure. Fractions less than 0.5 will be omitted and fractions greater than or equal to 0.5 will be rounded up to the next higher unit.

1.   Height and Weight

a)   Standing Body Height

The participant stands erect on the floor or the horizontal platform with his/her back against the vertical mounted ruler, heels together and against the vertical ruler, looking straight ahead with his/her head in the Frankfort horizontal plane (the horizontal plane which includes the lower margin of the bony orbit and the bony socket containing the eye the most forward point in the supratragal notch just above the anterior cartilaginous projections of the external ear) (Figure 1). The right angle is brought down snugly but not tightly on the top of the head. A foot stool is used if the examiner is shorter than the participant so that the examiner's view is level with the point of measurement on the head of the participant. The participant's height is recorded to the nearest centimeter using the rounding method described above. The participant is instructed to stand as straight as possible but with feet flat on the floor. (A check is made to be sure the floor is level, the wall is at a 90 degree angle to the floor, the wall is straight and the metal ruler is mounted perpendicular to the floor). A chart converting centimeters to inches is on the wall or available for use in informing the participant of his/her height in inches.

b)   Body Weight

Before a participant is weighed, the scale is balanced so that the indicator is at zero when no weight is on the scale. The scale must be level and on a firm surface (not a carpet). The participant is instructed to stand in the middle of the platform of the balance scale (Detecto, model 683-p) with head erect and eyes looking straight ahead. Record the results to the nearest kilograms using the rounding method described above. To maintain accuracy, the scale is zeroed daily and must be calibrated with a known weight (50 lbs.) every month or whenever the scale is moved. To calibrate the scale, check that the 50 lb. weight weighs 50 lbs. after zeroing the scale. Furthermore, the operator should make sure that an adult must weigh 50 lbs. more when standing on the scale holding the weight.
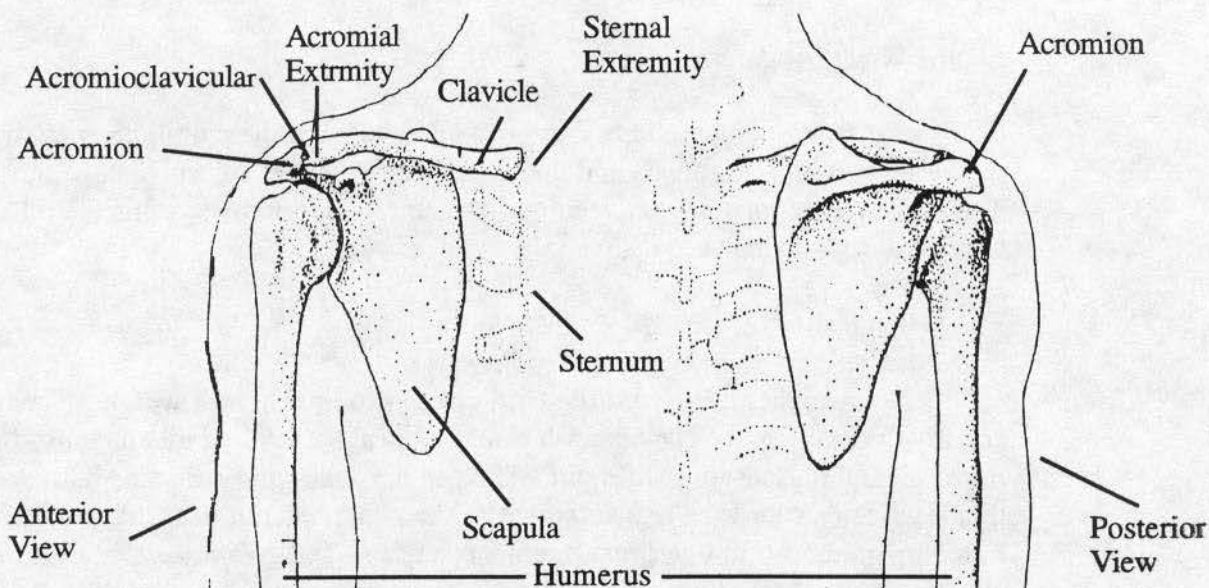
Figure 1 (a). General Description: The **scapulae**, or shoulder blades, are large, triangular, flat bones situated in the dorsal part of the thorax between the levels of the second and seventh ribs. A sharp ridge, the spine, runs diagonally across the posterior surface of the flattened, triangular body. The end of the spine projects as a flattened, expanded process called the **acromion**. This process articulates with the clavicle.
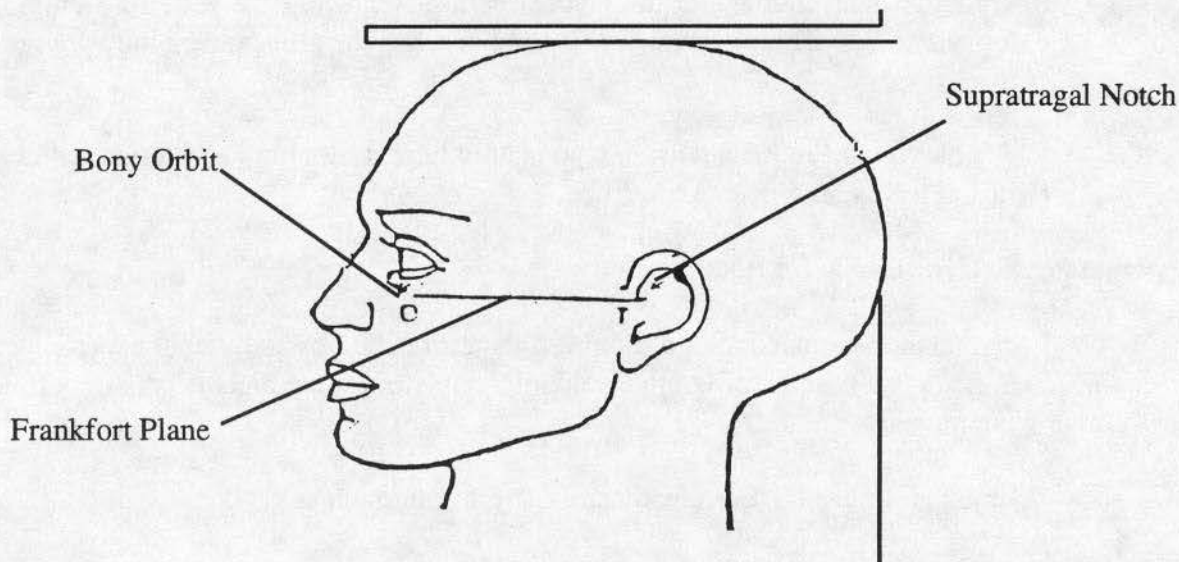
Figure 1 (b). **the Frankfort Plane:** The horizontal plane which includes the lower margin of the bony orbit, the bony socket containing the eye and the most forward point in the supratragal notch, the notch just above the small prominence of skin covered cartilage projecting over the meatus of the external ear.

2.   Supine Waist (Abdominal) Girth

An anthropometric tape is applied at the level of the umbilicus (naval) with the patient supine (Figure 2) and the participant is instructed to "breathe quietly". The measurement is made and recorded to the nearest centimeter using the rounding method described above.

3.   Erect Hip Girth

Instruct the participant to stand erect yet relaxed with weight distributed equally over both feet. The hip girth is measured at the level of maximal protrusion of the gluteal muscles (hips) (Figure 3). Keep the anthropometric tape horizontal at this level and record the measurement to the nearest centimeter using the above rounding method. Only one measurement is made. The greatest source of error for this measurement is due to not having the tape horizontal. Technician(s) should check the position of the tape to assure its correct position from both the front and back.

4.   Upper Arm Circumference

The participant sits on a table or stool so that the right arm hangs freely with the right hand resting on the right knee. The observer applies the tape measure horizontally at the midpoint between the acromium and olecranon (Figure 3). Record the measurement to the nearest centimeter using the rounding method described above. This measurement is used to select the proper size blood pressure cuff.

A Novel Products tension tape is used to measure both abdominal and hip girth and the upper arm circumference.

1.7.2 Training and Certification for Anthropometry

Each technician must undergo training and certification by individual experienced anthropometrist. The training program for taking body size measurements consists of the following components.

1.   Training is conducted centrally by an expert in anthropometry.

2.   Each field center trains one or two individuals before the baseline examination. One individual from each center is designated the center's anthropometry supervisor.

3.   If additional personnel are needed by a center to perform anthropometry, training is provided by the center's anthropometry supervisor.

4.   Training includes:

a.   Introduction - rationale for body size measurements, overview of technique, expected limits of reproducibility, and pitfalls related to anthropometry.
b.   Demonstration of technique - an expert demonstrates the proper technique of each measurement on a volunteer subject. This includes a description of proper and improper techniques, as well as recording of data.
c.   Practice - technicians divide into groups of three, and two techs perform measurements on the third in a round-robin fashion. This is done under the observation of a trained anthropometrist. Differences in technique and clarification of problem areas are discussed.
d.   Testing - several subjects are assessed independently and blindly by each technician. Each technician's measurements are compared with the expert's measurements and the results discussed in class. The four subjects examined have four distinctly different body types: lean, obese, athletic, and aged.
e.   Certification - technicians must measure one or more test subjects and be within certain standards of error:

1)   The arm, waist and hip measurements must agree within + 1 cm on each subject.
2)   Weight must agree within + 1 kg. Height within 1 cm.

If these are met, the subject receives certification for field work. Trainees who have problems are identified, and they are allowed to practice and try again to be certified.

1.7.3   Sitting Blood Pressure

1.   Introduction

        In the Strong Heart Study, sitting blood pressure is measured in a resting state, using 3 measurements with a Baum mercury sphygmomanometer. Within any one individual, variation in blood pressure is substantial, even within a few minutes and particularly under conditions perceived as stressful. Use of three replicate readings tends to reduce this short-term variation.

2.   Standardized Clinic Procedure

        Correct measurement of blood pressure is of the utmost importance to the success of this study. It is essential that the procedure described below for measuring blood pressure be followed exactly. Precision is essential for valid comparisons of blood pressure between groups of people and in individuals on different occasions.
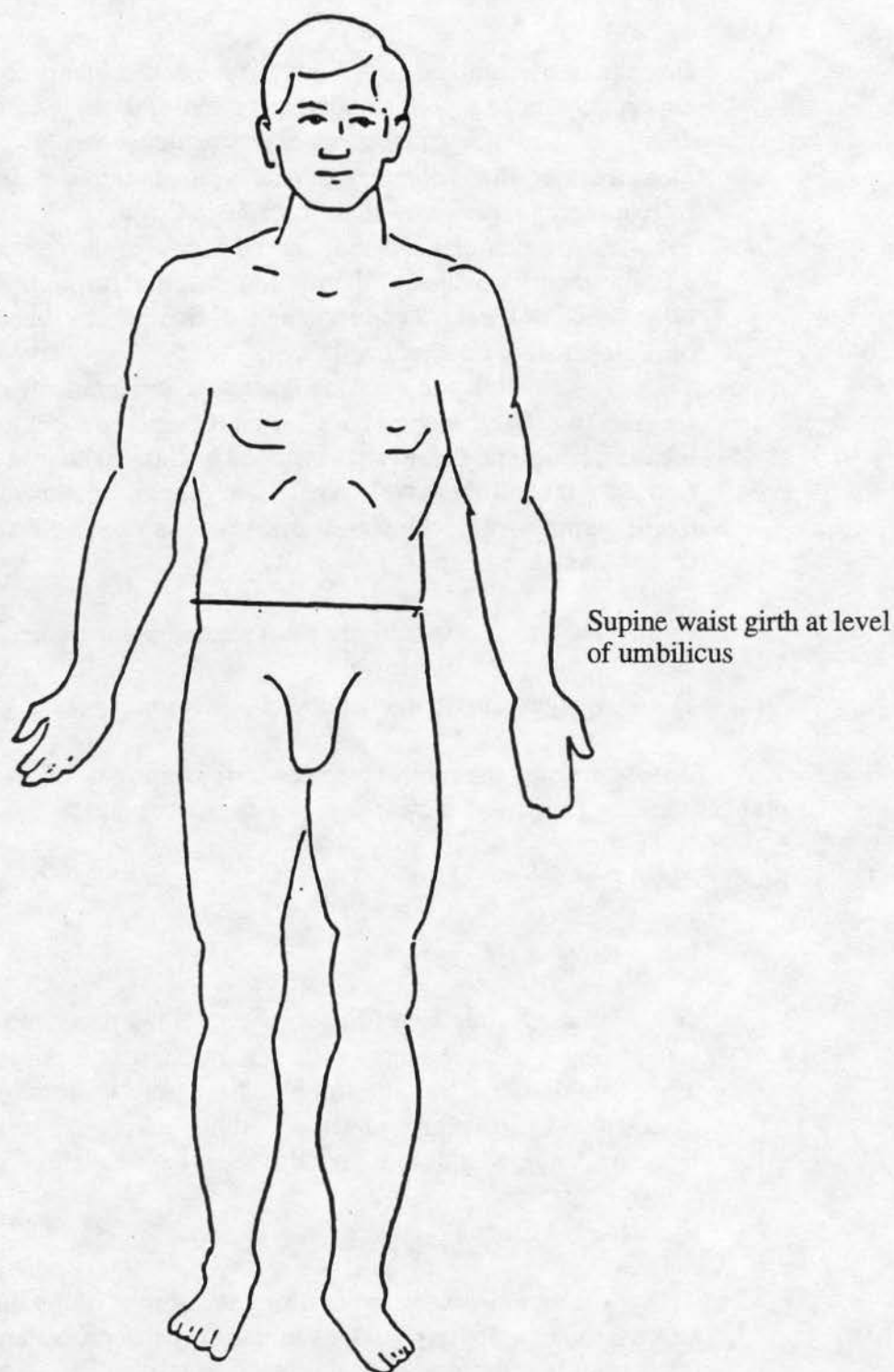
Supine waist girth at level of umbilicus

Figure 2. Location of Waist Girth Measurement
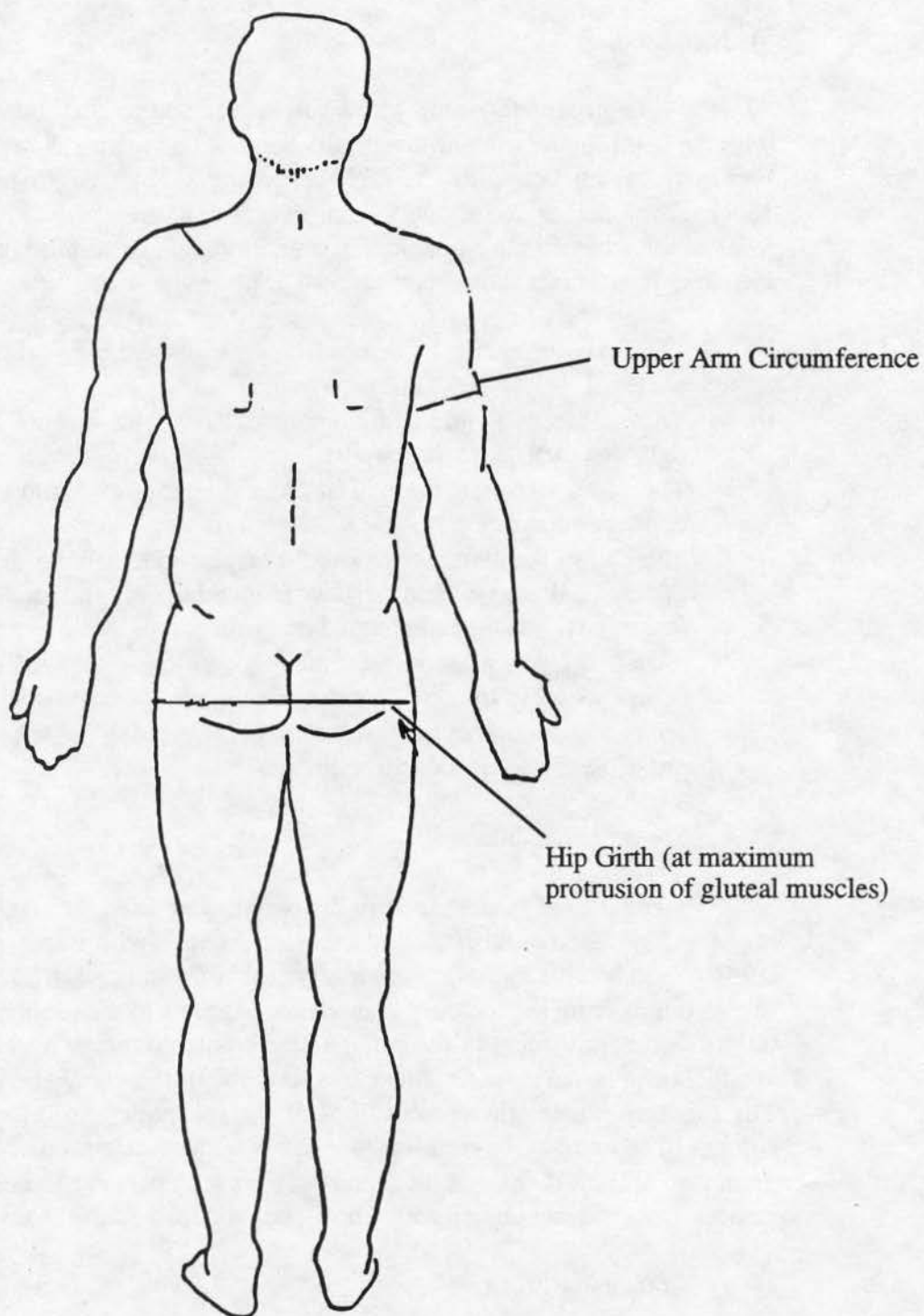
Figure 3. Location of Upper Arm, Hip, and Calf Circumference

3.   Description of the Equipment

a) Stethoscope

A standard stethoscope with a bell is used. Korotkoff sounds are best heard with the bell because of their low pitch. Stethoscope tubing should be about 10-12 inches from the bell piece to "Y" branching. This length provides optimal acoustical properties and allows the observer to read the sphygmomanometer at eye level and in a comfortable position. Earpieces should fit comfortably and snugly in the ears. Four points should be observed in using the stethoscope.

i)   The ear piece should be directed downwards and forwards into the external ear canal.
ii)   The ear pieces should be tight enough to exclude outside sound but not so tight that they cause discomfort.
iii)   The valve between the bell and the diaphragm should be turned in the correct direction.
iv)   The bell of the stethoscope should be placed lightly on the skin overlying the brachial artery - immediately below the cuff and medial to the cubital fossa above the medial epicondyle of the radius and posterior to the biceps muscle. Light pressure accentuates the low-pitched sound and avoids compression murmurs. When pressing too heavily with the bell on the artery a murmur can be heard which may prolong the apparent duration of phase 4 and give inaccurate readings.

b)   Sphygmomanometers

Standardized Baum mercury instruments are used for all clinic visits. The mercury manometer consists of a screw cap, a face with numbers, a lined glass column, a reservoir containing mercury, rubber tubing, and a metal case. The rubber tubing from the mercury manometer connects to the rubber tubing from the inflatable rubber bladder of the cuff. As the inflatable rubber bladder is filled with air, the air pressure in the bladder travels through the connecting rubber tubing. The pressure pushes the mercury out of the reservoir and into the lined glass column. The number for each line is read when the rounded top of the mercury, the meniscus, is level with it. If the meniscus is exactly between the lines, the reading is made from the line immediately above, i.e., rounded up the nearest even number.

c)   Cuffs and Bulbs

Proper size of the cuff is essential for accurate blood pressure measurement. Study Centers have three standardized Baum cuffs available - adult, large adult, and thigh cuff.

The range markings on commercial cuffs overlap from size to size and do not offer a precise guideline. In the Strong Heart Study arm size is measured and the cuff size is selected as follow:

Table 1.2 Determination of cuff size based on arm
          circumference (Mid humeral)

Cuff Size	Arm Circumference
Adult	24 to 32 cm
Large Adult	33 to 41 cm
Thigh	>41 cm

----

4.   Blood Pressure Measurement Instructions

Some of the many extraneous factors influencing blood pressure are controlled by standardizing the measurement technique and the environment in which the measurement is made. Uncontrolled factors, such as time of day, arm circumference, recent use of caffeine, and identity of the observer are recorded, so that they can be taken into account during analysis.

The Strong Heart Study participants are asked to avoid caffeine (tea, coffee, chocolate, and soft drinks), eating, heavy physical activity, smoking and alcohol intake for twelve hours and to refrain from smoking for at least one-half hour prior to the clinic visit. Current drug intake, including medications affecting blood pressure, and non-prescription drugs is recorded on the day of the examination. A detailed history of alcohol intake history and recent physical activity at work and leisure are also recorded.

5.   Staff Preparation for Participant Visit

In relating to the Strong Heart participants, remember that participation in the study is voluntary. Participants are given full explanation and instructions about the preparation for the blood pressure examination and an opportunity for questions. The setting in which blood pressure measurements are made is standardized.

6.   Measurement Procedures

The sitting arm blood pressure is measured three times at each clinic visit. It takes approximately 10 minutes to make three blood pressure measurements including the initial five minute rest. The blood pressure measurements are made early in the clinic visit sequence immediately following the reception and informed consent, and more than 15 minutes after phlebotomy.

Once the participant is given instructions and explanations, and the equipment has been checked, blood pressure measurement begins. The following steps must be followed precisely.

a)   If the participant indicates that there is a medical or postsurgical reason for not having the blood pressure measured on the right arm (or if the right arm is missing), reverse chairs and proceed with the left arm. Indicate on the data collection form that the left arm is used. If in doubt, or if the participant prefers not to have a blood pressure taken on either arm, consult with the supervisor.

b)   Seat the participant with the right arm on table. The bend at the elbow (ante-cubital fossa) should be at heart level. Legs should be uncrossed and head support comfortable. The participant should be able to relax the neck and shoulder muscles as much as possible.

c)   Connect the cuff to a standard manometer and establish the pulse obliteration pressure by slowly inflating while palpating the radial artery until pulse is no longer felt. Deflate and record the pulse obliteration pressure.

d)   Palpate the brachial artery (just medial to and above the ante-cubital fossa), and mark this location for stethoscope placement. Choose the correct cuff size and wrap the cuff on the arm with the center of the bladder over the artery. If the participant seems particularly apprehensive, delay wrapping the cuff until after the five minute wait.

e)   Record the time. Allow a five minute wait before taking the blood pressure. Conversation should be limited. However, a brief explanation of the procedure can be repeated at this time if necessary.

f)   Measurement 1: Connect the cuff to the manometer. Inflate rapidly to the pulse obliteration level + 30 mm. Holding the pressure constant with the bulb, wait 5 seconds. Place the bell of the stethoscope on the brachial artery

and slowly deflate the cuff (2 mm per second) while listening. Record the 1st and 5th phases, reading the pressure in mmHg to the nearest even number. The first sound heard in a series of at least two sounds is recorded for systolic blood pressure (phase 1). The first silence in a series of at least two silences is recorded for diastolic blood pressure (phase 5), not the last sound heard. If the sounds do not cease completely, the fourth Korotkoff sound will be used.

g) Measurements 2 and 3: Have the participant raise measurement arm for five seconds. After waiting another 25 seconds with the participant's arm on the table, repeat the measurement in step f above and disconnect cuff.

Average blood pressure readings are calculated for the second and third readings. Because of the importance of the blood pressure averages, to inform the participant and for the purposes of referral, all arithmetic is done with a calculator.

If for any reason the observer is unable to complete, or has forgotten to complete any portion of the examination (and the participant is gone), draw two horizontal lines through the space(s) on the form. This is the correct way to indicate missed information. If an entire reading is missed and the participant is still sitting at the blood pressure work station, completely deflate the cuff and start over with a replacement reading.

7. Reporting the Blood Pressure Results to the Participant

Using a calculator, average the second and third readings and mention the results to the participant. State clearly the systolic and diastolic pressure.

8. Procedure for changing the peak inflation level

Occasionally the Korotkoff sounds may be heard as soon as one places the stethoscope over the brachial pulse. If this happens, the peak inflation level used was too low. The observer immediately deflates the cuff by releasing the thumbscrew and disconnecting the cuff tube. Then have the participant hold the cuff-wrapped arm vertically for five seconds. Proceed with blood pressure measurement, starting at a new peak inflation level, 10 mmHg above the previous level.

9. Sitting Blood Pressure Training and Certification

At each field center a minimum of two clinic staff persons are trained for measuring sitting blood pressure. They need not be health professionals, but they

must be trained and certified in the blood pressure measurement technique. Observers should also have experience in relating to people.

The first training session begins with a description and demonstration of the correct blood pressure measurement procedure. Trainees watch the American Heart Association blood pressure instruction video tape. Checklist is used for certifying all persons taking BPs (Appendix 7 (a) & (b)). Simultaneous BPs will be recorded using a Y stethoscope as described in Appendix 9.

It is the responsibility of each field center to conduct these procedures and report to the Coordinating Center when the procedures are completed.

Y Tube stethoscope observations are made in conjunction with the blood pressure training video during initial training and for quarterly quality control. The trainer has the observer-trainee go through the entire blood pressure measurement procedure using a quality control checklist. The observer and trainer listen with the Y Tube and record the values on separate sheets. Two measurements on one subject are obtained. Measurements by the trainer and the trainee should agree within 4 mmHg on any one reading (systolic or diastolic) and averages should agree within 3 mmHg.

10.   Quality Control

To ensure the accuracy of the blood pressure measurements throughout the study, quality control measures are developed centrally and applied at all field centers. These measures include:

a)   recruitment of the most qualified personnel
b)   standardized training and certification
c)   retraining as necessary
d)   observation of data collection by supervisors, using the checklist given in Appendix 9. One checklist is used for each technician and mailed to the Coordinating Center each month
e)   frequent staff meetings to provide feedback
f)   editing of data, both manual and by computer
g)   a quality assurance program administered by the Coordinating Center
h)   simultaneous Y Tube observation of each technician by the blood pressure supervisor
i)   equipment and maintenance program

11.   Technician Training and Quality Control

Blood pressure technicians are trained centrally prior to participant recruitment. New technicians hired after the start of the study are trained locally by the Study Coordinator or a designated "Blood Pressure Supervisor".

The Coordinating Center directs a blood pressure quality assurance program to review six-monthly data. This includes quality analysis and review of blood pressure data every 3 months, comparing means for each technician with the values for all technicians, by center. These statistics are adjusted for weight, age and sex of the participants by the use of Z-scores. Arbitrary levels of Z-scores, (which can be modified according to performance) are used to detect possible systemic deviations in blood pressure measurement by individual technicians. Digit preference is also monitored for each technician. The Form for Recording Simultaneous Blood Pressure Observations in Appendix 9 will be used.

12.   Equipment Maintenance

Each study center is responsible for the proper operation and maintenance of its equipment. Maintenance responsibility is assumed by the nurse clinician, and all staff are instructed to report any real or suspected equipment problems to that person promptly.

All checks, inspections, cleanings and problems indicated are documented and recorded by date in a permanent log. Problems and solutions are also recorded. The local nurse clinician sends a copy of this log monthly to the Coordinating Center. A copy of this log is given in Appendix 10.

The standard sphygmomanometer is inspected once a month. These inspections include a check of:

i)   the zero level
ii)   mercury leakage
iii)   manometer column for dirt or mercury oxide deposit
iv)   condition of all tubing and fittings

The equipment is cleaned if inspection indicates it is needed, or at least once a year. Specific maintenance instructions for the standard sphygmo-manometer are provided in Appendix 11.

1.7.4 Ankle Systolic Blood Pressure

1.   Move the Participant to the Supine Position

Assist the participant in moving to the supine position on the examination table.

2.   Applying the Blood Pressure Cuff

The appropriate ankle blood pressure cuff is applied on the right calf. The same size cuff should be used on the ankle as the one used on the arm. In special instances, different cuff size may be used.

At this point, a blood pressure cuff is applied above the ankle of the right leg, as shown in Figure 4. Place the cuff flat on the table (the surface marked "side to the patient" face up) with the appropriate ankle centered on the cuff. At this time disregard the "over the artery" marker. The lower edge of the cuff (from which the hoses extend) should be approximately 2-2 1 2 inches above the medial malleolus. Following the contour of the lower leg, wrap the end of the cuff with the velcro "fabric" over the ankle, as shown in Figure 5. Note that depending on the degree of tapering in this area, the cuff corner will be offset from parallel toward the knee. Holding the cuff from sliding, wrap the other end over the ankle (step III in Figure 5), again following the contour of the ankle, and secure the velcro. Check to be sure that the corners of the cuff extending above the upper edge of the cuff are about equal: if one end extends more than the other, loosen the velcro and adjust the wrap. Next, locate the "over the artery" marker of the cuff, and rotate the cuff so that this line is directly over the posterior tibial artery. The cuff may be rotated more easily by sliding it toward the malleolus, and after alignment, the cuff can be made snug by pulling it up toward the calf. The cuff should conform closely to the shape of the ankle, with the lower edge 2-2 1 2 inches above the malleolus.

The posterior tibial artery is usually palpated as it courses posteriorly to the medial malleolus. Even if the posterior tibial pulse is not palpable, the posterior tibial artery is used as the location for the marker line on the cuff for the "over the artery position". Any kinks in the tubing are removed, and any "tugging" of the tubing on the participant's leg is relieved.

3.   Procedure for Measuring Ankle Blood Pressure

a)   Palpate posterior tibial pulse and mark these locations. Apply ultrasound gel to the posterior tibial area over the pulse or in the area shown on Figure 4.

b)   Listen for the pulse using the Imex Mascot Doppler. If no pulse is audible or palpable, Then try to use the dorsalis pedal pulse for the determination of blood pressure. If no pulse is audible, record zero for ankle blood pressure after the absence of pulse is verified by a second observer.

c)   Inflate cuff to a pressure reading 20 mm higher than the "Peak Pressure" used for the sitting arm pressure (i.e., obliteration plus 50 mmHg) and utilize identical deflation techniques while listening with the Doppler.

d)   Take a second blood pressure, and record both blood pressure in the Physical Examination Form. This procedure also applies to the doppler arm blood pressure.

e)   Record the first sound as described above.

The blood pressure cuff is applied over the brachial artery according to the instructions found in the Sitting Blood Pressure section of this manual. By consulting the participant's Data Form, the observer verifies that the same arm and the same cuff size are used as for the sitting blood pressure readings.

If the participant had his/her sitting blood pressure taken on the right arm earlier in the clinic examination, the cuff is applied on the right arm at this time. The observer then uses the Doppler to record the brachial pressure. The pressure recorded in the right arm is used to calculate the brachial/ankle systolic pressure ratio for both lower extremities.

If it is impossible to obliterate the sounds after increasing the pressure to above 250 mmHg, no systolic measurement should be made in that ankle.

f)   Repeat the procedure for the left leg and record the pressure as soon as the cuff is in the proper position.

The observer now removes all conduction jelly. Socks and a robe or other garments are now replaced and the participant is escorted to the next work station.

1.7.5   Electrocardiogram

1.   Basic description

a)   A Marquette Mac-PC (or Mac-12) based system will be used.

b)   All ECGs will be transmitted centrally to Fitzsimons Army Medical Center in Denver electronically by modem.

c)   All ECGs will be read in a standard manner at the ECG Reading Center by Board Certified or Board Eligible Staff Cardiologists and transmitted or mailed back to the site of origin for clinical correlation or other action, if required. In any case, all ECGs will be overread and promptly returned.

d)   All ECGs will be forwarded to the University of Minnesota ECG center for application of Minnesota codes.

e)   The Strong Heart Study will itself maintain a permanent copy of all cardiograms in its possession to assure "perpetual" availability of the study data for study members.

f)   A standard level of competence must be demanded of our personnel performing ECGs at each site. A "competency exam" would be conducted of all persons recording ECGs at individual sites by a physician (or other designated person) who would judge the ability of the person being examined to adhere to standard protocol.

2.   Minimal Equipment Requirements

a)   A new Mac-PC with modem will be used at each clinic. Mac-12 machines may be used if they are available.

b)   Fitzsimons Army Medical Center will provide free use of their mainframe CAPOC (Computer Associated Practice of Cardiology software) system (except for study hook-up costs and paper costs) for the duration of the study. This system can be accessed 24hrs/day by modem and stores all study cardiograms together or by center. Also, floppy disc downloading can be accomplished to a Mac-12 compatible format.

Transmission instructions on Mac-PC, Standardized ECG, instructions and Minnesota Codes are given in Appendices 33, 34, and 35, respectively.

Procedures will differ at each center concerning how ECG readings are supplied to local physicians and IHS health records. A copy of the ECG obtained at the time of performance, if marked "unconfirmed" can be included in the patients chart that day. A clinical reading will be performed at Fitzsimons and returned by reverse transmission procedure WITHIN one week. A hard copy of this clinical reading will also be sent to the Coordinating Center for storage.

All ECGs will be sent to the University of Minnesota to be coded. The Minnesota codes will then be added to the ECG data set by the Coordinating Center for data analyses.
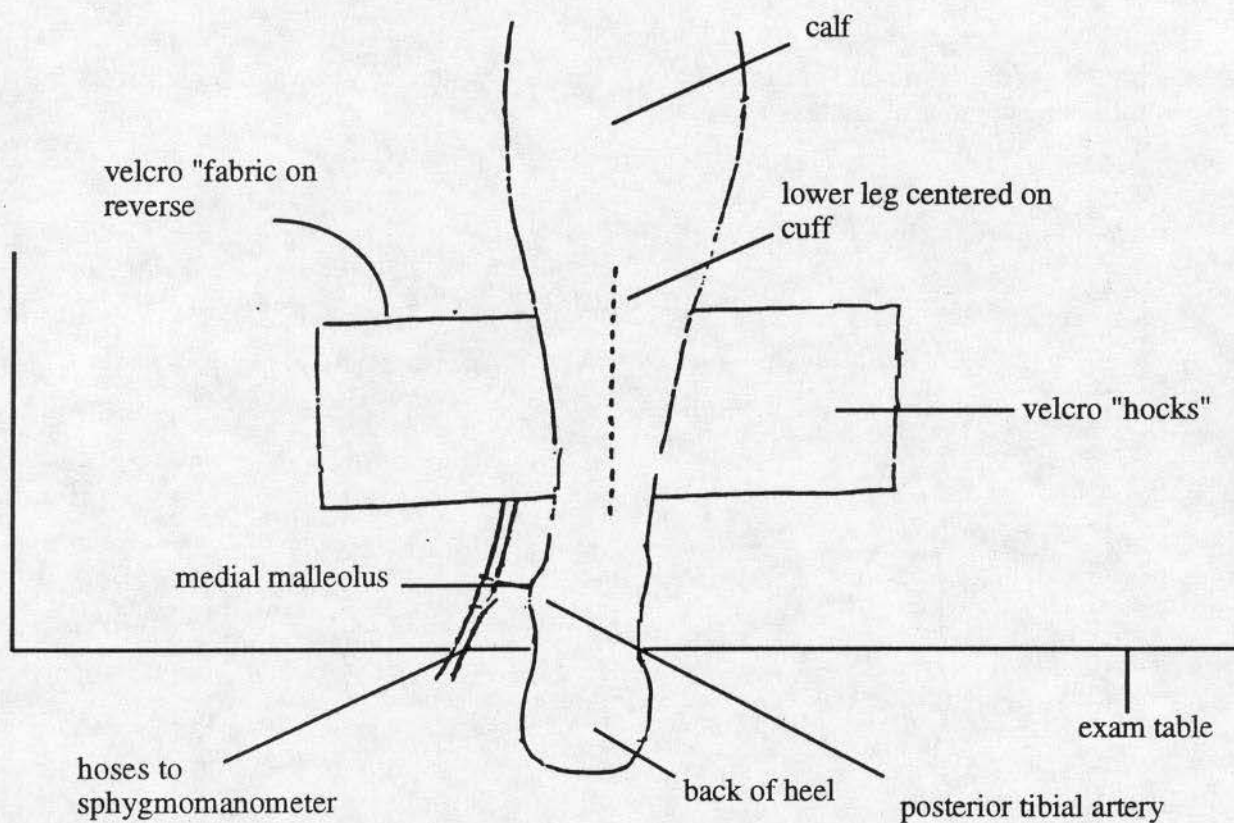
Figure 4. Placement of the blood pressure cuff on the ankle. Step 1 - Positioning the lower leg on the cuff.

Step 2. Wrap fabric end of the cuff
following contour of ankle
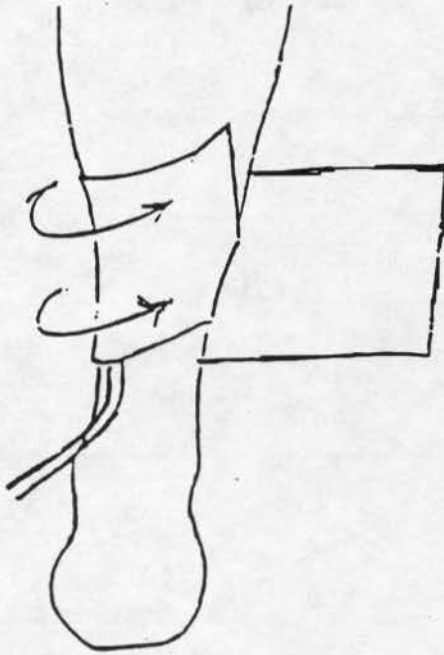
Step 3. Wrap and secure cuff

"ears" about
equal
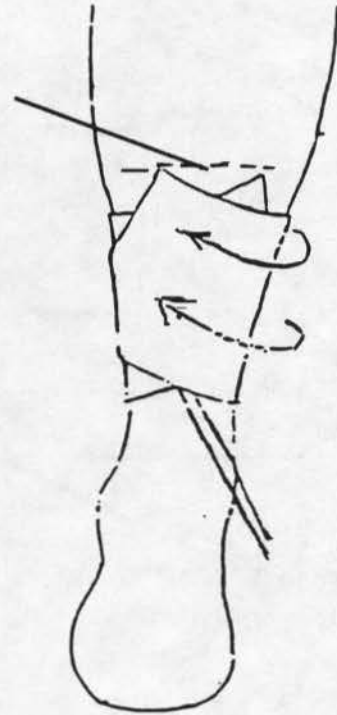
Figure 5.   Placement of the blood cuff on the ankle. Step 2 and Step 3: Wrapping and
securing the cuff

1.7.6 Impedance Measure

The measurement of body fat is accomplished using the Impedance Meter, Model # B1A101, made by RJL Equipment Company. This involves a small low frequency current which travels across the body through the extracellular fluids. The measurement of bioelectrical impedance is related to the volume of the conductor and when expressed as impedance or conductance, it is proportional to fat free mass.

1.   Procedure

a)   Before beginning explain why you are making the measurement to the subject and check to see that the subject has not exercised vigorously for the past 12-hours and has not consumed alcohol in the past 24-hours. Make sure that the subject is not dehydrated. Record past vigorous exercise or alcohol consumption on the data form.

b)   Before beginning the test, be sure that the subject cable is securely attached to the RJL spectrum, have the subject remove the right shoe and sock and lie down with the right side nearest to the analyzer;

c)   If the examination table is metallic, it must have a foam pad - all of the body must be on the pad.

d)   For best results:

i)   Use electrodes only once.

ii)   Legs should be far enough apart so that thighs do not touch each other. A towel may be used to prevent the legs and thighs being touching.

iii)   Hands and arms should be far enough apart so that the arms and hands do not touch the torso. A towel can be used to prevent the arms from touching the body.

iv)   No body parts should be in contact with any external metal (jewelry and pins in bones will not effect the results).

v)   Subject's skin should be clean, dry and warm to the touch. If the skin is oily, clean it with an alcohol swab before attaching the electrodes.

Prior to the attachment, cut the electrodes in half bisecting the foil tab. The cut edge of the electrode placed on the ankle and wrist should face toward the shoulder and thigh respectively. The cut edge of the other two may face in either direction.

e)   Electrode Placement:

i)   Attach the black wires to the foot with the red clip connected to electrode at ankle (F1). Attach the red wires to the hand with the red clip connected to electrode at the wrist (H1).

ii)   Put H1 on an imaginary line from the protruding bone of the wrist to bisect the ulnar head; make sure that the cut edge of the electrode is toward the shoulder.

iii)   Put H2 just above the knuckles of the right hand or on any finger; there should be at least 5 cm difference between H1 and H2.

iv)   Put F1 on an imaginary line between the protruding ankle bones to bisect the medial malleolus; make sure that the cut edge of the electrode is toward the thigh.

v)   Put F2 just above the toes of the right foot or on the great toe (there should be 5 cm difference between F1 and F2)

Once the electrodes have been properly attached to the subject, the values for resistance and reactance will appear on the screen. Record these on the results sheet. Make sure that the toggle switch is set on x1 .

2.   Instructions for Impedance Meter

Battery Charging

Unit has rechargeable batteries that must be charged before use. They are charged by plugging instrument in with power switch in off position. Manufacturer suggests charging for 8 hours prior to use. Instrument should not be plugged in longer than 8 hours; damage to batteries may occur.

For our use they suggest the following: Plug unit in first thing in the morning before clinic and at least 15 minutes before the first test. Leave unit plugged in for the duration of each clinic, but have the power on only when testing a patient. At the end of clinic, the meter should indicate high charge (green area).

Checking Instrument

Before testing the first patient, be sure that the cables are not crimped or damaged. Check battery charge using the following procedure. Disconnect power cord. Place the Resistance/Reactance switch in the resistance position. Place the switch labeled x1/x10 in the x1 position. Attach the 2 clips from one patient cable to one side of 500 ohm resistor provided.

Attach the two clips from the other cable to the other side of the resistor. Turn power on. Resistance displayed should be between 490 and 510 ohms. If resistance is in this range, proceed with patient testing.

If resistance is not within this range, the batteries may not be fully charged, or another problem may be present. If charge appears to be low, charge batteries for 8 hours, then retest. If unit is fully charged and resistance is still not acceptable, see manual, page 9, for trouble shooting.

Note: Patient cables are made of silver. Take care not to bend or abuse cables. They should be left plugged into instrument to minimize handling, except when relocating instrument.

3.   Quality Control for Impedance Measure

Training for the measurement of body fat using the bioelectric impedance meter was accomplished by a representative from the RJL Equipment Company who attended the training followed the following steps:

a.   Instructions concerning the use and verification of the machine.

b.   Demonstration by instructor of the procedure.

c.   Practice by the individual operators.

d.   Certification of operators if instructor and operator achieve an impedance measure where resistance and reactance were each within 3%.

For ongoing quality control in each center, one individual will be designated as supervisor of the impedance measures. This individual will assure that each of the other operators of the instruments is recertified quarterly by having him/her perform an impedance measure on the same individual as the instructor. These should agree within 3%.

In addition, the instructor is responsible for the monitoring of the impedance meter. This includes checking the battery charge daily before the instrument is used, following the

instructions in the manual. Further, the instructor should observe individual operators performing impedance measures at least quarterly to verify consistent and proper technique.

1.7.7   Examination of the Lungs, Bruits and Pulse

1.   Sitting Examination

Neck - Presence or absence of venous column in 90 o upright position is recorded.

2.   Sitting or Supine Examination

Neck, Carotid Bruits - The participant is asked to stop breathing momentarily. With the stethoscope bell, the examiner listens first above the clavicle for the common carotid artery and second, at the angle of the jaw for carotid bifurcation. In each position, the stethoscope is placed for three cardiac cycles, alternating sides of the neck.

3.   Auscultation

Auscultation can be performed while patient is sitting quietly waiting (five minutes) for blood pressure measurements.

a)   Lungs

Lungs - Rhonchi, Rales- The participant is in the sitting position. It may be best for men to remove the scrub top or shirt entirely and for women to lift it. The stethoscope diagram (which should be warmed in the palm of the hand) is used. The participant is instructed to take deep breaths through the mouth. After the first five or six breaths and as needed thereafter, the participant is asked about symptoms of light-headedness. Auscultate posteriorly beginning at the apices with at least one full breath in each location. Three locations on each side are examined: apex, mid-lung field (approximately at the 6th intercostal space) and the base, which may need to be determined by percussion. Rhonchi are described as coarse breathing noises.

Rales are fine moist noises. Basilar rales are reported as those within two stethoscope diameters of the base of the lung. "Lower lung" means from above the base to mid-lung, at the 6th space posteriorly.

4.   Supine Examination

a)   Femoral Bruits

The femoral artery should be auscultated by stethoscope using the diaphragm at the inguinal crease bilaterally for the evidence of bruits. (This is a large artery readily palpable in all but the most obese individuals).

b)   Ankle Edema

The socks or other foot covering are removed. The participant is examined in the supine position. Gentle but firm pressure is applied along the mid-tibia, anteriorly down to the ankle in each leg. Pitting or indentation remaining after pressure is removed constitutes definite edema. The examiner identifies the mid-point between the prominence of the medical malleolus and the inferior border of the patella. Pitting at or above that mid-point is recorded as "marked" edema. Pitting only below that point is recorded as "mild" edema. The degree of edema is based on the extent.

c)   Posterior Tibial Pulse

The examiner palpates inferior to the medial malleolus of each foot. The presence or absence of arterial pulsation is recorded. If in doubt, the examiner compares with the radial pulsation.

d)   Dorsalis Pedis Pulse

The superior aspect of each foot is palpated for the presence or absence of this pulse.

e)   Peripheral neuropathy using monofilaments.

1.7.8   Physical Findings to be Confirmed by a Physician to Assure for Presence of CHF

The participants should be referred for evaluation by a physician if these findings were noted so they can be confirmed and evaluated for the presence of congestive heart failure.

1.   Lung Examination

Rales in at least three of four lower lung field in the sitting position.

2.   Other findings

Bilateral ankle edema, orthopnea or paroxysmal nocturnal dyspnea.

The standard IHS referral form should be used to refer patients with newly observed physical findings described above to an internist or cardiologist so that the diagnosis can be confirmed and the prevalence of congestive heart failure can be determined. In such cases, use the Physician Referral Form for Diagnosis of CHF in Appendix 14.

1.7.9   Referral Guidelines

It is the intention of the Strong Heart Study that individuals who participate in the physical examination will be provided both with education and encouragement concerning a healthy life style aimed at preventing cardiovascular disease and, also that the participants receive assistance in securing medical care for any significant medical conditions uncovered during the course of the study exam.

1.   Referral procedure:

a)   All participants reporting for the medical exam will receive appropriate educational materials concerning a heart health lifestyle. In addition, the examining personnel, when possible, will endeavor to educate the participants during the exam concerning the importance of risk factor reduction and modifications that the individual might make to improve his/her risk for cardiovascular disease.

b)   After all laboratory results are completed from the physical examination, a follow-up letter will be mailed to each participant thanking him or her for participation and supplying him/her with basic medical information obtained during the exam. (See example of letter and suggested interpretation in Appendix 15).

c)   After all results from the medical examination are complete, a form will be generated by the Coordinating Center which will be available to the Indian Health Service for insertion into the patients medical record. This will contain results of the electrocardiogram, measurements of body fat, glucose tolerance test, and blood measurements, which might be of benefit for their future medical care.

d)   In order to insure that the patient receives appropriate referral and treatment for significant medical conditions uncovered during the course of the study, consistent referral levels have been established as described below which will be applied at each center.

2.   Referral Levels and Medical Data Review

The Strong Heart Study refers participants using established guidelines for referral. Uniform criteria for referral of participants are implemented at all centers. Emergency, immediate, urgent, and routine referrals are made. Methods for referring participants who

have no physician are established with the participant. All referrals are documented on a separate log and copies of the referrals are kept in the Strong Heart Study folders. The following are the levels of referral established for the Medical Data Review.

a)   Emergency Referral: The patient is immediately escorted to a physician or an emergency squad is summoned. In such situations study personnel will provide emergency care to the best of their ability and training as appropriate to the emergencies that arise.

b)   Immediate Referral: The participant is urged to see his/her physician within one day.

        The physician assistant/nurse clinician notifies the participant's physician or nearest IHS facility and the Strong Heart Study physician, if applicable. The participant is provided with an IHS referral form to take to his/her physician.

c)   Urgent Referral: The participant is urged to see his/her physician within one week.

        The physician assistant/nurse clinician confirms the decision with the Strong Heart physician, if applicable, of the referral. An IHS referral form is filled out and an appointment is made with the assistance of the clinic staff and/or CHRs.

d)   Routine Referral: The participant is asked to see his/her physician within one month, or at first convenient appointment.

        The physician assistant/nurse clinician advises a visit to the participant's physician. Appointments for the patients are made by the CHRs or clinic staff.

e)   No Referral: The study results are summarized for participant and held for routine results letters.

3.   Referral and Review Guidelines for Independent Patient Follow-up

Guidelines for referral at medical data review are provided in the table below. The reviewer determines the acuteness of the findings, as well as whether or not the condition is being followed by a physician.

If the participant is aware of and being followed medically for a condition, judgement is exercised about whether to refer. The standard IHS referral form is used to provide appropriate clinical information to the health care professional who will evaluate the patient. A copy of this referral will be retained with the research forms to document the referral that was made.

Referral at the time of examination

Emergency Referral	Statement to Participant ("Consult M.D. immediately")
SBP ≥ 260 mmHg DBP ≥ 130 mmHg	Your BP is very high Your BP is very high
Pulmonary edema or any finding or symptom suggestive of a life-threatening illness, including evidence of acute MI. Use Referral form (Appendix 14)	Describe rationale for referral to participant

Immediate Referral	Statement to Participant ("Consult M.D. today")
Fasting One Touch glucose > 400	Your blood sugar is very high
SBP 240-259 mmHg DBP 115-129 mmHg	Your BP is very high Your BP is very high
Diabetic foot ulcer	Your foot must be seen by a physician
Unstable angina	Your chest pains may be important
Neurologic symptoms in past week	Your symptoms may be important
Other severe symptoms or findings	Your symptoms may be important
Echocardiogram finding: Pericardiac tomponade Intercardiac mass	You may have serious problem in your heart

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SBP 200-239 mmHg	Your BP is high
DBP 105-114 mmHg	Your BP is high
Angina, stable but untreated/not being followed	Your chest pains may be important
Neurologic symptoms, untreated, one week to six months ago	Your symptoms may be important
Suspected congestive heart failure (Use Referral Form in Appendix 14)	Your symptoms may be important
Other acute, but less severe symptoms	Your symptoms may be important
Inappropriate medication usage may be dangerous	Taking medication incorrectly
Non-diabetic with a fasting One Touch glucose of ≥ 200	Your blood sugar is high
Pulmonary function test findings: Undiagnosed severe pulmonary disease	You may have serious problem in your lungs
Clinic cough, fever, weight loss, and other symptoms suggestive of active TB or valley fever	You may have serious problem in your lungs
Echocardiogram: Previously undiagnosed potentially show stenosis	You may have serious problem in your heart
Gallbladder suspicious for cancer	Your symptoms may be important

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Routine Referral	("Consult M.D. within one month or at first convenient appointment")
SBP 140-199 mmHg	Your BP is elevated into borderline range. Recommend that participant confirm blood pressure reading within 2 months
DBP 90-104 mmHg	Your BP is elevated into borderline range. Recommend that participant confirm blood pressure reading within 2 months
Old MI (Rose Questionnaire), previously unrecognized	Your chest pain may be important
Neurologic problem (stroke, TIA findings) > 6 months ago, unrecognized	Your symptoms may be important
Claudication, previously unrecognized	Your leg pain may be important
Both pedal pulse are missing in one extremity and not previously referred or the ratio of doppler pressure of ankle/arm < 0.8	You may have a problem in your arm of foot. You should check with your doctor
Carotid Bruit: previously undiagnosed	You have a heart murmur and carotid murmur and should be checked by your doctor
Undiagnosed peripheral neuropathy	Your symptoms may be important
Undiagnosed moderate pulmonary disease	Your symptoms may be important
Mild valvular disease	You may need antibiotics when you have dental work
symptomatic gallstone	Results from reading center to be reviewed by physician

Referral after results are available	Consult M.D. within 1 month (If critical values: FBG>400, TG>1000, creatinine>2, cholesterol >300, laboratory will call field center)
Fasting blood glucose $\geq$ 140 or 2 hr-glucose $\geq$ 200, and non-diabetic	You may have diabetes
Fasting blood glucose $\geq$ 200 and diabetic	Your diabetes is not under control
2-hr blood glucose 140-199	Your blood sugar is high. You may develop diabetes
Cholesterol > 200	Your blood cholesterol must be rechecked
Triglycerides > 1000	Your triglycerides is very high
Triglycerides > 250	Your triglycerides must be rechecked
Plasma Creatinine > 2 and no previous history of kidney problems	Your kidneys are not functioning well
Urine Albumin > 1000 mg/day or Plasma creatinine 1.5-2.0	Your urine test shows you should be checked
Undiagnosed valvular disease	Results from reading center to be reviewed by physician
Positive PPD and no previous therapy or preventive therapy	Refer participant to preventive therapy

ECG REFERRAL: <u>ECG Findings Requiring Review by M.D. before Participant leaves the clinic</u>

Would like to review with M.D.,
Call should be made to Reading Center by field staff at (303) 361-8133

*   Acute pattern abnormalities (MI, ischemia)

*   Rhythm disturbances
              2nd or 3rd degree block, ventricular tachycardia,
              any type of ectopic beat > 6/minute, couplets bigeminy, R on T,
              multifocal premature ventricular contractions,
              atrial fib/flutter with ventricular rate < 60/min or > 110/min,
              sinus bradycardia < 40/min, sinus tachycardia > 110/min, PR interval $\geq$ 0.26 sec.

*   Any other ECG findings, alone or in conjunction with symptoms, causing concern

<u>Other ECG Findings to be reviewed the same day; if possible</u>

QT Prolongation (confirm medications)

<u>ECGs where Routine Referral is usually appropriate</u>

New left bundle branch block
New right bundle branch block
Wolff Parkinson White
Left Ventricular Hypertrophy

Examples of Usually Benign ECGs (always obtain old comparison ECG when available)

Left Axis Deviation/Left Anterior Hemi (Fascicular) Block
Atrial Abnormalities, Intraventricular Conduction Delay
Unusual P Wave Axis, Wandering Atrial Pacemaker
S_1 S_2 S_3 Pattern, Old Right Bundle Branch Block
Incomplete Right Bundle Branch Block
ST Elevation compared with Early Repolarization
First Degree AV Block

Copies of each ECG obtained as part of the Strong Heart Study will be forwarded to either
the local clinical director or other identified local clinical personnel.

1.8   QUALITY CONTROL

1)   Anthropometry and blood pressure

Duplicate measures of arm blood pressure (systolic and diastolic), ankle blood pressure, and anthropometry (height, weight, waist/hip ratio, and electrical impedance measurements) should be performed by a second observer on an approximate 10% randomly selected sample of participants. These data must be sent to the Coordinating Center for monthly analysis. Results of the analysis will be provided to the field centers and the Steering Committee on a monthly basis. Criteria for unacceptable differences are as follows:

1)   Systolic Blood Pressure: 15 mmHg
2)   Diastolic Blood Pressure: 15 mmHg
3)   Height: 1 cm
4)   Weight: 0.5 Kg
5)   Resistance: 15 units

Duplicate data for blood pressure, height, and weight will be compiled by the Coordinating Center and reported to the clinics and Steering Committee quarterly; in addition, distributions of measurements and digit preference for each staff member will be compiled and repeated quarterly.

Anthropometric measurements and blood pressure by standard sphygmomanometer and by Doppler should be observed and evaluated quarterly by the clinic supervisor. This person will also assure that each of the other operators of the impedance meter is recertified quarterly by having him/her perform an impedance measure on the same participant as the supervisor. In addition, a simultaneous Y-tube observation of each observer by the blood pressure supervisor should be made. All results will be analyzed by the Coordinating Center on a quarterly basis. Duplicate blood pressures taken by Doppler will be performed quarterly by the supervisor.

To maintain accuracy, the scale should be zeroed daily and should be calibrated with a known weight (50 lbs.) every month or whenever the scale is moved. The impedance meter should be calibrated daily, follow manufacturer's instructions. This includes checking the battery charge daily before the instrument is used. The standard sphygmomanometer should be inspected once a month. These inspections include a check of (i) the zero level, (ii) mercury leakage, (iii) manometer column for dirt or mercury oxide deposit, and (iv) condition of all tubing and fittings. Record equipment monitoring or a checklist. The Coordinating Center will compile the data and document staff performance.

2)   Laboratory tests

        Duplicate blood and urine specimens should be collected on approximately 10% of the participants and sent to the Core Laboratory in a blind fashion. Results obtained for each test will be analyzed monthly by the Coordinating Center for accuracy and consistency. The percent of pairs with differences within 5%, 10%, and 20% will be computed. Correlation coefficients and coefficients of variation will be calculated and technical errors estimated. Poor correlation or unreasonably high technical error will be reported to the Laboratory and the Steering Committee.

3)   Personal interview

        Personal interviews must be observed monthly by the study coordinator. Problems and errors should be identified using a checklist and corrected immediately.

4)   Pulmonary function tests and echocardiography

        Refer to PFT section and Echo section, respectively.

5)   Data quality at the Coordinating Center

        As in Phase I, every data form received from the field centers should be checked for completeness by staff at the Coordinating Center. If there are any apparently incorrect entries or missing data, the staff will contact the respective field center for clarification. The entire form will be returned to the field center if the problem can not be solved by telephone. After all the questionable items are clarified, the form will be entered and verified by two different persons.

        The data entry program provides a second quality control check. Range checks and logical checks should be built into the data entry program. The program will refuse to accept such data until the errors are corrected. Computer printouts of data received will be sent to each field center. Summary statistics such as mean, median, range, maximum and minimum for continuous variables and frequency distributions for categorical variables will be calculated monthly for each center and data not meeting consistency checks will be flagged.

6)   Quality control for surveillance data

        In the mortality and morbidity surveys, decisions on cardiovascular disease (CVD) deaths and CVD events of interest will be made by the three physicians on the Mortality and Morbidity Review Committee. Duplicate records of every death and 10% of all morbid events will be sent to a second member. Each physician independently determines the classification of a cause of death or CVD event, and the Coordinating Center then compares

the results from both physicians. If the primary reviewer feels a decision on the cause of death or that regarding a possible CVD event is particularly debatable, all information will be sent to another reviewer so that a joint decision can be reached. The committee will meet annually to discuss equivocal cases.

The Mortality Committee will also evaluate the quality of chart reviews and advise clinic staff if changes are needed.

7)   Quality control site visits

Quality control site visits will be scheduled every six months. The site visit team which consist of the Program Manager from NHLBI and representatives from every center will visit each center, observe every component of the study, identify inconsistencies, discrepancies, and other problems, and provide advice for improvement.

8)   Certification of technicians

Each center will recruit the most qualified personnel. Clinical staff will be centrally trained and certified before the examination begins and newly hired personnel are trained at each clinic. Recertification occurs every six months to ensure accurate and consistent performance.

9)   Confidentiality and safety of data

All personnel with access to data collected for the study are required to sign a confidentiality pledge. Completed data forms are placed in locked file cabinets at every center and only authorized staff members have access to the data.

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APPENDIX 1
THE STRONG HEART STUDY II

UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER

Individual's Consent for Participation in a Research Project

I, _____, voluntarily agree to participate in the study entitled:
Subject

CARDIOVASCULAR DISEASE IN AMERICAN INDIANS (The Strong Heart Study Phase II), which is sponsored by the National Heart, Lung, and Blood Institute, under the supervision of Dr. Elisa T. Lee.

I understand:

1.   **PURPOSE:** The purpose of this study is to assess how often cardiovascular disease (disease of the heart) occurs in American Indians and who are more likely to develop heart disease.

2.   **STATUS OF INVESTIGATIONAL DRUG/DEVICES/PROCEDURES:** There are no investigational drugs/devices/procedures involved in this study.

3.   **DESCRIPTION OF STUDY:** I will be given a physical examination, which includes the following procedures: weight, height, girth measurements (measurements of the arm, waist, and hip), blood pressure, measurements of body fat using an impedance meter, a resting electrocardiogram (ECG), an echocardiogram, a pulmonary function test, a tuberculin skin test, and an ultrasonogram of the gallbladder. The impedance meter involves several electrodes being placed on my foot and the back of my hand. The electrocardiogram is to test whether my heart is functioning normally. Several electrodes will be placed on my chest with an ointment. The echocardiogram is a test that involves the use of sound waves to look at my heart and the blood vessels around my heart. The echocardiogram is done with a small probe being moved around on the surface of the skin on my chest. For the pulmonary function test, I simply have to exhale into a small plastic tube. The tuberculin skin test involves the injection of a small amount of tuberculin under the skin of my forearm (bubble test) to determine if I have been exposed to tuberculosis. The gallbladder ultrasonogram involves the use of sound waves to detect whether there are gallstones in my gallbladder. A blood sample will be taken from my arm to measure the amount of lipids (fat) and other substances in the blood. A small drop of blood will be taken from a finger prick to measure my fasting glucose level by One- Touch. If it is less than 225 mg/dl, I will be given a glucose tolerance test, that is, I will be asked to drink a sweet beverage containing 75 grams of carbohydrate; and two hours later another blood

sample will be taken from my arm to measure how well my body can tolerate the sweet drink. If I have diabetes and I am on insulin or I am on oral agents and I have had two glucose measurements 250 mg/dl or higher, I will not be given the glucose tolerance test. A total of up to four ounces of blood may be taken from my arm, and a small amount will be stored for future studies, including genetic factors that may be related to health and illness. A urine sample will also be taken. I must have fasted for at least 12 hours prior to the physical examination.

In addition to the physical examination, I will answer some questions on diet, stress, smoking and drinking habits, exercise activity, use of any medications, heart disease history, and any other medical problems. The entire examination and interview will take about two and one-half hours. I agree to allow the results of the examination and any information in my medical record to be used for statistical purposes to further medical knowledge. I understand that any medically important information will be included in my Indian Health Service medical record, and I may request and authorize, by signature, its release to other agencies or persons as I feel appropriate.

At the physical examination, if problems are found that require immediate attention, I will be referred to the Indian Health Service for appropriate care. The Strong Heart Study will not be able to pay for follow-up tests or treatment recommended.

4. **BENEFITS:** I understand that my participation may prove beneficial in advancing scientific knowledge regarding heart disease. I also may benefit from this physical examination and evaluation. I will be reimbursed $25 for my travel expenses.

5. **POSSIBLE RISKS:** Possible risks/side effects include discomfort and bruising, bleeding, fainting, and infection from blood drawing and possible discomfort from the impedance meter and electrocardiogram (ECG) measurement, which includes having electrodes (small suction cups or adhesive pads) placed on my chest when partially unclothed and lying still for approximately ten minutes. For the echocardiogram and pulmonary function test, there are no risks/side effects involved, with the possible exception of slight dizziness in some people after the pulmonary function test. These risks/side effects would not be more than those which could occur in a good routine physical examination.

If I have any side effects, I will report them immediately to Dr. Lee or her staff. If side effects are severe, which is unlikely, I may be removed from this study.

6. **IN THE EVENT OF INJURY, INFORMATION CONCERNING MEDICAL TREATMENT AND COMPENSATION:** In the unlikely event of injury, established as a result of my participation in the research, appropriate short-term medical treatment will be provided by the Indian Health Service. Neither the Indian Health Service, the Federal Government, nor the University of Oklahoma has provisions for financial compensation in the event of such injury.

7. **FOLLOW-UP:** You will be sent Strong Heart Study newsletters on a periodic basis throughout the study and after it is over to inform you of the results of the study. Study investigators or their colleagues may recontact you for further information about your health in the future.

8. **CONFIDENTIALITY:** I also understand that information obtained will be treated as confidential, and no personal information or name will be made public in any form.

9. **SUBJECT ASSURANCE:** By signing this consent form, I acknowledge that my participation in this study is voluntary. I also acknowledge that I have not waived any of my legal rights or released this institution from liability for negligence.

   I may revoke my consent and withdraw from this study at any time without penalty or loss of benefits. My relations with the physician(s) and staff at the Indian hospitals or clinics, now and in the future, will not be affected in any way if I refuse to participate, or if I enter the program and withdraw later.

   If I have any questions or need to report an adverse effect about the research procedures, I will contact the Principal Investigator, Dr. Elisa Lee, or colleagues by calling (405) 271-2229 or 271-3090 during a workday.

   If I have any questions about my rights as a research subject, I may take them to the Director of Research Administration, University of Oklahoma Health Sciences Center, Room 115, Library Building, telephone number (405) 271-2090.

10. **SIGNATURES:** I have read this informed consent document. I understand its contents, and I freely consent to participate in this study under the conditions described in this document. I understand that I will receive a copy of this signed consent form.

   _____   _____
   Date   Signature of Research Subject

   _____   _____
   Date   Signature of Witness

   _____   _____
   Date   Signature of the Principal Investigator

APPENDIX 2 (a)

THE STRONG HEART STUDY II

Clinical Examination -- Checklist

Participant's name: _____

                          Last   *First*   *Middle*

ID Number: [_____]   Date: [□□/□□/□□]

                                                                        mo   day   yr

	Items	If done, date and initial
1.	Consent Form Signed	_____ _____
2.	Medical Release Signed	_____ _____
3.	One Touch blood test, Reading _____	_____ _____
4.	ProAct/Reflotron (if done), Reading _____	_____ _____
5.	Fasting blood sample	_____ _____
6.	Glutol	_____ _____
7.	Urine sample	_____ _____
8.	Two-hour blood sample	_____ _____
9.	Skin test	_____ _____
10.	Personal interview forms	_____ _____
11.	Medical history form	_____ _____
12.	Medical chart review to identify morbidity cases	_____ _____
13.	ECG	_____ _____
14.	Impedance measurement	_____ _____
15.	Height and Weight	_____ _____

16. Abdominal, hip and arm circumference   _____ _____

17. Sitting blood pressure   _____ _____

18. Doppler blood pressure   _____ _____

19. Examination of lungs and vessels   _____ _____

20. Neuropathy tests   _____ _____

21. Echocardiogram   _____ _____

22. Gallbladder - ultrasound   _____ _____

23. Pulmonary function test   _____ _____

24. Dietary survey   _____ _____

25. Psychosocial questionnaire   _____ _____

26. Quality of life questionnaire   _____ _____

27. Payment or payment form   _____ _____

APPENDIX 2 (b)

STRONG HEART STUDY

POST EXAM ACTIVITIES

Same Day:

Process blood specimens
Review morbidity (chart review at clinic site)
Stamp patient's clinic chart with SHS exam information
Add codes: community, tribe, clinic/hospital, medicines
Edit for missing data
Transmit ECG's to Denver
Make all but routine referrals
Complete ECHO measurements
Clean pulmonary function apparatus

Later:

Send ECHO, gallbladder and pulmonary function tapes to reading centers
Make routine referrals
File confirmed ECG and ECHO reports
Mail letters to patients
File laboratory findings in patients medical records
Xerox questionnaires for data center
Mail data to Oklahoma
Mail laboratory specimens
Verify skin tests

APPENDIX 2 (c)
Flowchart for Physical Examination

```
                    ┌─────────────────────────────┐
                    │ Registration, Consent Form  │
                    └─────────────────────────────┘
                                   │
        ┌──────────────────────────────────────────────────────────┐
        │ Measure glucose by One Touch and draw fasting blood sample │
        └──────────────────────────────────────────────────────────┘
                                   │
┌──────────────────────────────────────────┐
│   Is patient                               │            yes
│ 1. On insulin or                           │
│ 2. On oral hypoglycemic agents             │     ┌──────────────────┐
│    and had 2 previous glucose              │     │  Urine Specimen  │
│    values  250 mg/dl or                    │     └──────────────────┘
│ 3. Fasting One Touch  225 mg/dl            │              │
└──────────────────────────────────────────┘
              no                                   ┌──────────────────────────┐
                                                   │ Personal Interview, dietary│
                                                   │ survey can be done 2 weeks │
                                                   │ before or after exam and   │
                                                   │ blood draw.                │
┌──────────────────────────────────┐              └──────────────────────────┘
│ Administer 75 gm glucose load    │
└──────────────────────────────────┘              ┌──────────────────────┐
              │                                    │ Weight, height,      │
                                                   │ circumferences       │
┌──────────────────────┐                          └──────────────────────┘
│ Examine  lungs        │
│    and vessels        │
└──────────────────────┘                          ┌──────────────────────┐
                                                   │ Blood pressure       │
┌──────────┐                                       │ at least 15 minutes  │
│  ECG     │───────────────────────────────────────│ after blood draw     │
└──────────┘                                       └──────────────────────┘

┌──────────────────────────┐
│ Impedance measurement    │──────────────────────────
└──────────────────────────┘

┌──────────────────────────────┐              ┌──────────────────────┐
│ Echo (heart, gallbladder)    │──────────────│ Pulmonary function   │
└──────────────────────────────┘              └──────────────────────┘

┌──────────────┐                              ┌──────────────────────┐
│ Skin tests   │──────────────────────────────│ Neuropathy tests     │
└──────────────┘                              └──────────────────────┘

                    ┌─────────────────────────────┐
                    │ Draw 2-hour blood sample     │
                    │ (For subjects who had the    │
                    │   glucose load only )        │
                    └─────────────────────────────┘
                                   │              ┌──────────┐
                                   │──────────────│  Snack   │
                                   │              └──────────┘
                    ┌─────────────────────────────────┐
                    │ Educational material, Payment or │
                    │ Payment paper, Thank You         │
                    └─────────────────────────────────┘
```

APPENDIX 3
THE STRONG HEART STUDY II
CARDIOVASCULAR DISEASE IN AMERICAN INDIANS

PERSONAL INTERVIEW FORM I

ID number:

Community name: _____   Community Code: ☐☐☐

Social Security Number: ☐☐☐ - ☐☐ - ☐☐☐☐

A.   DEMOGRAPHIC INFORMATION:

1.   What is your full name (*Last, middle, first*) and date of birth?

Last: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Middle: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

First: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Have you changed your name since SHS-I examination? (1=Yes, 2=No)   ☐

Date of birth (mo/day/yr)   ☐☐ / ☐☐ / ☐☐

2.   To which IHS and non-IHS Hospital/Clinic do you usually go? *List the one they go to most often first. Give names and codes.*

	Hospital	Chart number	IHS (1=Yes, 2=No)	Hospital Code
a.	_____	☐☐☐☐☐☐	☐	☐☐☐☐☐☐
b.	_____	☐☐☐☐☐☐	☐	☐☐☐☐☐☐
c.	_____	_____	☐	☐☐☐☐☐☐
d.	_____	_____	☐	☐☐☐☐☐☐
e.	_____	_____	☐	☐☐☐☐☐☐
f.	_____	_____	☐	☐☐☐☐☐☐

3.   What is your husband's/wife's name? (*If divorced or widowed, draw two lines over boxes*)

Last: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Middle: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

First: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

4. Did he/she also participate in the Strong Heart Study examination? □
   1=yes,   2=no

5. Did any of your relatives also participate in the Strong Heart Study examination?
   1=yes   2=no   9=unknown.   If yes, please tell us his/her name:

   Relatives   Name (*first, last*)   yes/no □

   Parents   _____   □

   Brother   _____   □

   Sister   _____   □

   Children   _____   □

   Other blood relative _____   □

6. What is your current mailing address?

   a. Street/PO Box   [ ]

   b. City/town   [ ]

   c. County   [ ]

   d. State and zip code   [ ][ ]—[ ][ ][ ][ ][ ]

7. What is your residential address? (*If different from mailing address*)

   a. Street Number   _____

   b. City/town   _____

   c. State and zip code _____

8. What is your home or evening telephone number and area code?
   (*Draw line through boxes if no phone*)   [ ][ ][ ]—[ ][ ][ ]—[ ][ ][ ][ ]

9. What is your work or daytime telephone number and area code?(*Draw line through boxes if
   no phone or if it is the same as above*)   [ ][ ][ ]—[ ][ ][ ]—[ ][ ][ ][ ]

10. Where do you want your Strong Heart Study results sent?   □

    1=your current mailing address (*Q6*)   2=Other, specify:_____

    The address to which the SHS results should be sent: _____
                                                                    Street #

    _____   _____
           Street   *Apt.#*

    _____   _____
           City   *State - Zip Code*

ID number:

Social Security Number:

11. What is your marital status?
     Enter up to 3 options with the most recent one in the left most box.
     1= never married   4= separated
     2= currently married   5= widowed
     3= divorced
     6= POSSLQ (Person of Opposite Sex Sharing Living Quarters)

B.   TOBACCO:

12. Do you smoke tobacco for ceremonial purposes ?
     1 = yes   2 = no   9 = unknown

13. Do you smoke cigarettes now?
     1= yes   2= no (*Skip to Question 20*)

14. On the average, how many cigarettes do you usually smoke a day?
     Indicate the number of cigarettes smoked daily
     00= Less than one cigarette per day
     99= Unknown

15. Would you like to quit smoking cigarettes?
     1=yes   2=no

16. Do you plan to make any changes in your smoking cigarettes habit in the next 12 months?
     1=yes   2=no (*Skip to Question 17*)

     If "YES," which of the following are you planning to do?
          1=Quit completely
          2=Try to quit
          3=Cut down on number of cigarettes smoked
          4=Switch to lower "tar" or "nicotine" cigarettes
          5=Other, specify: _____

17. During the past year have you quit smoking cigarettes?
     including short term attempts for one day
     1=yes   2=no

     If "YES," how many times in the past 12 months have you attempted to
     quit AND were able to stay off cigarettes for a week or more?

18. Has a doctor or health professional ever advised you to quit smoking cigarettes? □
    1=yes   2=no

19. Have you participated in one or more quit smoking programs in the past 12 months? □
    1=yes   2=no

CURRENT CIGARETTES SMOKERS SKIP TO WEIGHT CONTROL QUESTIONS

20. How many years ago did you quit smoking ?   □□
    00 = never smoked 100 cigarettes during lifetime.

21. Did you quit smoking in the last 5 years?   □
    1=yes,   2=no (*skip to SECTION C*)

    If quit in last 5 years ask:
    a.   Before you quit, how many times did you attempt to quit and   □□
          were able to stay off cigarettes for a week or more?

    b.   What was the main reason you quit? (*choose one only*)   □
          1=Doctor's advice   2=Health concerns
          3=Expenses   4=Per family pressure
          5=Other, specify: _____

    c.   When you finally quit smoking, did you quit with outside help or □
          on your own?
          1=with outside help, how: _____
          2=on my own

C. WEIGHT CONTROL: The next few questions are about efforts to lose weight.

22. Are you now trying to lose weight :   □
    1 = yes   2 = no (*Go to Section D*)
    8 = unknown/unsure   9 = refused

23. Are you eating fewer calories to lose weight ?   □
    1 = yes   2 = no   8 = unknown/unsure   9 = refused

24. Have you increased your physical activity to lose weight ?   □
    1 = yes   2 = no   8 = unknown/unsure   9 = refused

25. Has a doctor or health professional ever advised you to lose weight ? □
    1=yes   2=no

D.   ALCOHOL:
"The next few questions are about the use of wine, beer, or liquor, including all kinds of alcoholic beverages".

READ THE FOLLOWING TO THE PARTICIPANT:
"We are asking these questions about alcohol, because we think alcohol consumption may be related to heart disease. We want to assure you that this information is strictly confidential and that we are not trying to judge your drinking habits and do not intend to report them to anyone."

26.   How long ago did you last drink any kind of alcoholic beverage?
Indicate number of days, months, or years since their last drink.

Number of days _____
(*if they drank today, fill in zero in days*)
       OR
Number of months _____
(*if they drank this month, fill in zero in months*)
       OR
Number of years _____
(*if they drank this year, fill in zero in years*)
If NEVER DRANK ALCOHOL, fill in 88 . If one or more years, skip to Question 32.

27.   How many drinks of alcoholic beverages do you have in a typical week?
Enter 1 for occasional drinkers.

        1 qt. of beer = 2.5 drinks
        1 pt. of beer = 1.5 drinks
        1 pt. of wine = 4 drinks
        1 qt. of wine = 8 drinks
        0.5 gal. of wine = 16 drinks
        1 pt. of hard liquor = 12 drinks
        One-fifth of hard liquor = 19 drinks
        1 case of beer (12 oz. cans) = 24 drinks
        6 pack of beer (12 oz. cans) = 6 drinks

Add up the total number of drinks in a typical week and fill them in the box in Question 28. Round up to nearest whole number if fraction is greater than or equal to 0.5.

28.   On how many days in a typical month do you have at least one drink?
Indicate number of days per month.

29.   On the days when you drank any liquor, beer or wine, about how many drinks do you have on the average ? *Indicate number of drinks per day.*

30.   How many times during the past month did you have 5 or more drinks on an occasion?
Indicate times per month.. Enter zero if subject has quit drinking more than one month ago.

31. How many times during the past year did you have 5 or more drinks on an occasion? *Indicate times per year. Enter zero if subject has quit drinking more than one year ago.*

E. PERCEIVED STRESS

In the **past month**, how often have you (*Questions 32-38*):
(1=Not at all   2=Rarely   3=Sometimes   4=Often   5=Most of the time)

32. been upset because of something that happened unexpectedly?

33. felt nervous or "stressed"?

34. dealt well with irritating life hassles?

35. felt that things were going your way?

36. felt unable to control irritations in your life?

37. felt that you were on the top of things?

38. felt difficulties or problems were piling up so high that you could not handle them?

F. PHYSICAL ACTIVITY

39. Since the last SHS exam have you ever spent any time confined to a bed or chair for greater than one month as a result of an injury or an illness?
1=yes   2=no (*Go to Question 41*)

40. If "Yes," how many **months** did confinement to a bed or chair last?

41. Have you had any difficulty getting in or out of a bed or chair? (1=Yes, 2=No)

42. During a typical day (including time spent both at work and at home), how long do you usually spend,

   a)   sleeping at night?   Hours: ☐☐   Minutes: ☐☐

   b)   napping during the day?   Hours: ☐☐   Minutes: ☐☐

   c)   walking ?   Hours: ☐☐   Minutes: ☐☐

   d)   carry/lifting moderate or heavy loads   Hours: ☐☐   Minutes: ☐☐
        (including children)?

43. Did you change your physical activity since the first Strong Heart exam ?
1=yes   2=no

   If "Yes,"   1=increased   2=decreased

G.   BOARDING SCHOOL

44.   Did you ever attend boarding school?   □
       1=Yes   2=No (*Skip to Question 46*)

45.   If "Yes," for how many years? (*Enter number of years*)   □□
       99=Not applicable

H.   DENTURE AND EATING PROBLEMS

46.   How many natural teeth do you have?   □
       1=all   2=most
       3=some   4=none

47.   Describe how you eat (*Choose ONE*):   □
       1=I use natural teeth to eat.
       2=The natural teeth I have don't help me eat at all
       3=I have natural teeth and a denture or partial. I use them both together to eat.
       4=I use dentures to eat.
       5=I chew with my gums.

48.   Rate your ability to chew food (*Choose ONE*)   □
       1=Good   2=Fair   3=Poor

I.   FAMILY INCOME:

49.   Which of the following categories best describes your annual **household**   □
       income from all sources? *Please show a list.*

       1= less than 5,000   6= 25,000 to 35,000
       2= 5,000 to 10,000   7= 35,000 to 50,000
       3= 10,000 to 15,000   8= over 50,000
       4= 15,000 to 20,000   9= don't know/not sure
       5= 20,000 to 25,000   0= refused

J.   ADMINISTRATIVE INFORMATION:

50.   How reliable was the participant in completing the questionnaire?   □
       1= very reliable   4= very unreliable
       2= reliable   5= uncertain
       3= unreliable

51.   Interviewer   □□□

52.   Date   □□/□□/□□
              mo   day   yr

Instructions for the Personal Interview Forms I and II

Subject should be seated comfortably and made to feel welcome during this interview because it is the first form collected and will set the scene for later data collection.

ITEM #   DESCRIPTIONS
==

Personal Interview Form I

Study Identification Number should be completely filled in with the number assigned at the time the consent form is completed and subject is registered.

> 1st digit represents the center number (1=SD, 2=OK, 3=AZ).
> 2nd digit should be "0" for all interviewees.
> 3-6 digits will be the consecutive number of the subject interviewed within the center.

Write in community code from list.

Write in social security number.

A.   Demographic Information

1   Enter last name, left justified.
      Enter first name, left justified.
      Enter middle name, left justified. If no middle name, leave blank.

2   Write down the name of IHS and the non-IHS hospital which subject usually goes. Write in facility with which number is associated.

3   Write down the name of participant's spouse.

4   Ask whether her/his spouse also participated in the Strong Heart Study.

5   Ask whether any of her/his first degree relatives also participated in the Strong Heart Study.

6a   Enter left justified with blank separating number from street name and street name from unit number. If post office box, enter after street address.

b   Enter left justified, city/town or reservation of residence.

c   Enter left justified, county of residence.

d   Enter state of residence as two digit postal abbreviation.
       AZ= Arizona   SD= South Dakota
       OK= Oklahoma   ND= North Dakota

7   If residential address is different from the mailing address, write in the residential address following the rules given in item 7a-d.

8   Enter complete telephone number of home phone or phone at which subject can be reached during the evenings.

9   Enter complete telephone number of work phone or phone at which subject can be reached during the day.

10   Ask the participant where does she/he want the Strong Heart Study results sent.

Note: All Personal Interview Forms I should be sent to the Coordinating Center separately for confidentiality.

Personal Interview Form II

11   Subject should be asked if he/she has ever been married. If yes, are you currently married? If not, are you divorced, separated or widowed? If a person had a common law marriage and now the partner is deceased, and the individual lives alone check widowed.

B.   TOBACCO These questions are very important to assess accurately because smoking is a major risk factor for cardiovascular disease.

12   This will determine how common ceremonial uses of tobacco is. Ceremonial tobacco use is probably not associated with adverse health effects.

13   Determine whether participant currently smokes cigerettes.

14   Determine average cigarettes smoked per day, which may have a significant effect on heart diseases and other health problems.

15-19   Are questions regarding quitting smoking. These will help IHS and tribes plan for smoking cessation program for this age group.

20   Determine when non-smoking participants quit smoking or if they are "never smokers" (smoked less than 100 cigarettes per day)

21a-c   Are questions for individuals who quit smoking in the last 5 years so we can learn how

they succeeded in quitting.

C. WEIGHT CONTROL: questions about efforts to lose weight

22-25 Ask participant whether she/he tried to lose weight, how and whether a doctor or health professional advised the participant to lose weight. These questions will provide useful information on the participants and health care providers are responding to the problem of obesity.

D. ALCOHOL Questions related to alcohol consumption are frequently not answered accurately in surveys. Questions included in this questionnaire have been widely used and validated in several national studies.

26 Question 26 determines when the individual last had any alcoholic beverage. If the last drink has less than 30 days fill in the box labeled number of days. If the last drink was within the last year, but more than 30 days fill in the number of months. If the last drink was over one year ago fill in the number of years. If the last drink was one or more years ago, skip to Q32.

27 Question 27 assesses the average number of drinks consumed in a typical week. Frequently individuals with severe drinking problems especially binge drinkers do not consume alcoholic beverages by the can, glass or shot, but rather drink wine or hard liquor out of a bottle. In these situations the interviewer must skillfully inquire as to the volume of each type of liquor consumed and then convert these according to the following table.

> 1 qt. of beer = 2 .5 drinks
> 1 pt. of beer = 1.5 drinks
> 1 pt. of wine = 4 drinks
> 1 qt. of wine = 8 drinks
> 0.5 gal. of wine = 16 drinks
> 1 pt. of hard liquor = 12 drinks
> One-fifth of hard liquor = 19 drinks
> 1 case of beer (12 oz. cans) = 24 drinks
> 6 pack of beer (12 oz. cans) = 6 drinks

Add up the total number of drinks in a typical week and fill them in the box in Question 28.

28 Question 28 will tell you the frequency of alcoholic consumption. Many individuals with severe alcohol problems will only drink on the weekends (i.e., 8 days per month) or at the time of the month when they receive income. Assume 30 days a month.

29-31 Question 29-31 are utilized to quantitate binge drinking. Use the information described above to assist in calculating number of drinks for individuals who drink bottles of liquor

or wine, rather than drinks. The alcohol consumption history can be validated by calculating the average drinks per month utilizing two different calculations as follows:

Question 27 drinks per week X 4 = drinks per month

Question 28 days per month drinking X Question 29 drinks per day also should give you drinks per month.

Question 30 quantitates binge drinking that occurs less frequently than once a month. For heavy binge drinkers, estimate annual number of binges for 1 month and multiply by 12.

E.   PERCEIVED STRESS

32-38 Stress has been associated with the occurrence of CVD in many population studies. Questions 32-38 assess the personal feeling about the degree of stress the SHS participant had in a general sense during the **PAST MONTH**. A cue card which lists all the answers should be used to help the participant to choose their responses.

F.   PHYSICAL ACTIVITY

39-41 Ask if the participant had been confined to bed or chair since last SHS examination because of injury or illness. If the answer is YES, fill in the number of months confined to bed or chair in Q40, and whether any difficulty getting in or out of a bed or chair in Q41.

42 a-d Ask, on the average, how much time the participant spent of sleeping, napping, walking and doing moderate/heavy work during a typical day.

43   Ask prticipant whether he/she had changed the amount of physical activity since last SHS examination.

G.   BOARDING SCHOOL:

44-45 Ask participant had ever attended boarding school and for how many years he/she attended.

H.   DENTURE AND EATING PROBLEMS

46   Ask participant how many natural teeth he/she still have.

47   Ask participant to describe how he/she eat.

48   Ask participant to rate his/her ability to chew food.

I   FAMILY INCOME

49   Question 49 assess the family income so that the subject's socioeconomic status can be determined. Ask the questions as stated in the questionnaire. Prepare a sheet of income levels to show the participant.

50   Question 50 assesses the reliability of the answers responded by the subject. Write down your personnel code number and the date of completion of interview.

ID Number: [   ]

Social Security Number: [   ]-[ ]-[   ]

A. MEDICATIONS - Prescription and Over-the-Counter

1. **Medication Reception:** As you know, the Strong Heart Study will be describing all medications its participants are using, both prescription and over-the-counter. These include pills, dermal patches, eye drops, creams, salves, and injections, as well as vitamins, cold or allergy remedies, aspirin, and Tylenol. We have asked you to bring all your current medications. Have you brought them with you? Are these all the medications that you took in the last two weeks? □

   1=Yes (*May I see them?*)
   2=Took no medicines (*Go to Question 3*)
   3=No (*Make arrangements to obtain or review Medical Record*)
   9=Refused, give reasons: _____ (*Go to Question 3*)

2. **Prescription Medications:**

2a. *Copy the name of the medicine, the strength in milligrams (mg) and the total number of doses prescribed per day (week or month). (Include pills, dermal, patches, eye drops, creams, salves, and injections.)*

Medication Name *Print the first 20 letters only - please print clearly*	Strength(mg) *write the decimal as one of the digits*	NDC Code (*For SHS Coordinating*	Class Code *Center Use Only*)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

12

13

14

15

Number unable to transcribe: _____ _____

3.   Over-the-Counter Medications:

□

3a.   Do you take any over-the-counter medications?
1=yes   2=No (*Skip to next section*)

3b.   *Copy the name of the medicine, the strength in milligrams (mg) and the total number of doses prescribed per day (week or month). (Include pills, dermal, patches, eye drops, creams, salves, and injections.)*

Medication Name	Strength(mg)	NDC Code	Class Code
Print the first 20 letters only - please print clearly	*write the decimal as one of the digits*	*(For SHS Coordinating Center Use Only)*	

1

2

3

4

5

6

7

8

9

10

Number unable to transcribe: _____ _____

Comments: _____

_____

_____

THE STRONG HEART STUDY II

MEDICAL HISTORY FORM

ID number:

Social Security Number: ☐☐☐–☐☐–☐☐☐☐

B. MEDICAL CONDITIONS:
"Now I'd like to ask you some questions about medical problems. Has a medical person EVER told you that you had any of the following conditions? "

4. High blood pressure?
    1=yes   2=no   9=unknown ☐

    If "YES," how old were you when you were first told by a medical person that you had high blood pressure? *Indicate the actual age.* Don't know=99 ☐☐

5. Arthritis?
    1=yes   2=no   9=unknown ☐

6. Cancer, including leukemia and lymphoma?
    1=yes   2=no   9=unknown ☐

    If "YES," specify type of cancer: _____

7. Diabetes?
    1=yes   2=no   3=borderline 9=unknown ☐

    If "YES" or "BORDERLINE," do you still have it now?
    1=yes   2=no   3=borderline 9=unknown ☐

    How old were you when you were first told by a medical person that you had diabetes? *Indicate the actual age.* Don't know=99 ☐☐

    What type of treatment are you taking for your diabetes? (1 = yes,   2 = no)

    a.   insulin ☐

    b.   oral hypoglycemic agent ☐

    c.   by dietary control ☐

    d.   by exercise ☐

    e.   do nothing ☐

8. Kidney failure?
    1=yes   2=no   9=unknown   □

    If yes, do you still have it now ?
    1=yes   2=no   9=unknown   □

    How old were you when you were first told by a medical person that you   □□
    had kidney failure? *Indicate the actual age.* Don't know=99

9. Renal dialysis?   □
    1=yes   2=no   9=unknown

10. Kidney transplant?   □
    1=yes   2=no   9=unknown

11. Cirrhosis of the liver?   □
    1=yes   2=no   9=unknown

12. LUNG PROBLEMS
    a.   Emphysema?   1=yes 2=no 9=unknown   □

    b.   Hay fever?   1=yes 2=no 9=unknown   □

    c.   Chronic bronchitis?   1=yes 2=no 9=unknown   □

    d.   Asthma?   1=yes 2=no 9=unknown   □

         If "YES" for asthma, do you still have it now?   1=yes 2=no 9=unknown   □

13. Have you had a heart catheterization?   □
    1=yes   2=no

    A heart catheterization is a study in which a tube is inserted into the heart through the groin
    or arm to see how the heart works.

    If "YES," which type of catheterization have you had and when?

    a.   Angioplasty?   (1=yes 2=no)   □

         If "YES," when and where ?   □□/□□/□□
         (*record the most recent*)   mo   day   yr

         hospital/clinic: _____

    b.   Other,   (1=yes 2=no)   □

         Specify: _____

         If "YES," when and where ?   □□/□□/□□
         (*record the most recent*)   mo   day   yr

         hospital/clinic: _____

14. Have you ever had an exercise test or Treadmill test to check your heart? ☐
    1=yes   2=no   9 = unknown

    If "YES," when and where?   ☐☐／☐☐／☐☐
    (record the most recent)   mo   day   yr

    hospital/clinic: _____

15. Have you had an electrocardiogram (ECG) taken since the last SHS examination? ☐
    1=yes   2=no   9 = unknown

    If "YES," when and where?   ☐☐／☐☐／☐☐
    (record the most recent)   mo   day   yr

    hospital/clinic: _____

SINCE your last SHS exam, that is ____(mo)____(yr), has a doctor told you that you had any of
the following conditions ?

16.   Heart failure ?   1=yes   2=no   9=unknown   ☐

       If "YES," when and where ? (If more than one episode since Exam I, enter information
       for THE FIRST ONE in the Exam I - Exam II interval)   ☐☐／☐☐／☐☐
                                                                mo   day   yr

       hospital/clinic: _____

       If "YES," do you still have heart failure now ?   ☐
       1=yes   2=no   9=unknown

17.   Heart attack ?   1=yes   2=no   9=unknown   ☐

       If "YES," when and where ? (If more than one episode since Exam I, enter information
       for THE FIRST ONE in the Exam I - Exam II interval)   ☐☐／☐☐／☐☐
                                                                mo   day   yr

       hospital/clinic: _____

18.   Any other heart trouble ?   ☐
       1=yes   2=no   9=unknown

       If "YES," specify type: _____   ☐

       If "YES," when and where ? (If more than one episode since Exam I, enter information
       for THE FIRST ONE in the Exam I - Exam II interval)   ☐☐／☐☐／☐☐
                                                                mo   day   yr

       hospital/clinic: _____

19. Stroke ?
    1=yes   2=no   9=unknown   □

    If "YES," when and where ? (*If more than one episode since Exam I, enter information for THE FIRST ONE in the Exam I - Exam II interval*)

    □□/□□/□□
    mo   day   yr

    hospital/clinic: _____

20. Enter information for multiple events.

    Reason: 1=Heart attack/heart surgery   2=Heart failure
                3=Other heart trouble   4= Stroke

	Hospital/Clinic	Town/State	Date (mo/day/yr)	Reason
i.			/ /	
ii.			/ /	
iii.			/ /	
iv.			/ /	
v.			/ /	

RESPIRATORY QUESTIONS

21. a. Do you usually have a cough?
        1=Yes   2=No (*Skip to Question 23*)   □

    b. Do you usually cough as much as 4 to 6 times a day, 4 or more days
        out of the week?
        1=Yes   2=No   □

    c. Do you usually cough at all on getting up, or first thing in the morning?
        1=Yes   2=No   □

    d. Do you usually cough like this on most days for 3 consecutive months
        or more during the year?
        1=Yes   2=No   □

    e. How long have you had this cough?   years: □□   months: □□

22. Do you usually bring up phlegm from your chest when you cough?   □
    1=Yes   2=No

23. Does your chest ever sound wheezy or whistling :

    a.   when you have a cold?   (1=Yes   2=No)   ☐

    b.   occasionally apart from colds?   (1=Yes   2=No)   ☐

    c.   most days?   (1=yes   2=No)   ☐

    d.   most nights?   (1=Yes   2=No)   ☐

24. Have you ever had an attack of wheezing that has made you feel short of breath?   ☐
    1=Yes   2=No

25. Are you troubled by shortness of breath when hurrying on the level or walking up
    a slight hill?   ☐
    1=Yes   2=No *(Go to Question 30)*   3=unable to walk *(Go to Question 30)*

26. Do you have to walk slower than people of your age on the level because of
    breathlessness?   ☐
    1=Yes   2=No

27. Do you ever have to stop for breath when walking at your own pace on the level?   ☐
    1=Yes   2=No

28. Do you ever have to stop for breath after walking about 100 yards (the length of
    a football field) or after a few minutes on the level?   ☐
    1=Yes   2=No

29. Are you too breathless to leave the house or breathless on dressing or undressing?   ☐
    1=Yes   2=No

30. Did you have any lung trouble before the age of 16?   ☐
    1=Yes   2=No

31. Have you ever been told you snore?   ☐
    1=Yes   2=No

IF THE PARTICIPANT IS MALE, GO TO ROSE QUESTIONNAIRE
IF THE PARTICIPANT IS FEMALE, GO TO NEXT PAGE AND CHECK HERE _____

THE STRONG HEART STUDY II

Procedures and Tests Photocopy Check List

ID Number

	Field Staff		Study Physician
	Copies Complete (√ when done)	Procedure Date (mmddyy)	Result (1=normal; 2=abnormal)
1. Cardiac Catheterization: Cath. report and pictures	_____	_____	_____
2. Treadmill test: ECG paper from test and report	_____	_____	_____
If thallium: also copy typed nuclear medicine report	_____	_____	_____
3. Other type of heart test: Type:_____ Reports report	_____	_____	_____
4. Bypass, angioplasty, balloon treatment or other heart surgery: Operative report	_____	_____	_____
5. Narrative Hospital Summary	_____	_____	_____

Abstractor Code

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REPRODUCTION AND HORMONE USE (WOMEN ONLY)

ID number: ☐

"The following questions are related to your childbearing organs".

1. Did you breast feed your last child for at least one month ? ☐
   1 = yes   2 = no   3 = never had a living baby

2. Have you ever been told that your blood sugar was high during any of the ☐
   pregnancies ?
   1 = yes   2 = no   3 = never been pregnant

3. Have your menstrual cycles stopped permanently ? ☐
   1= yes   2=no (*go to Question 6*)

4. How old were you when your periods stopped completely ? *Indicate the age in years.* ☐☐

5. Was your menopause natural or did you have surgery ? ☐
   1= Natural   2= surgery

   If surgery, was only your uterus removed? (1=yes 2=no 9=unknown) ☐

6. Have you ever used birth control pills? ☐
   1= yes   2= no

"ESTROGEN is a female hormone that may be taken after a hysterectomy or menopause."

7. Except for birth control pills, have you ever taken estrogen (*either pills, as a patch or* ☐
   by shot) for any reason? (Often called premarin: maybe either purplish brown or yellow
   football shaped pills once a day)
   1= yes   2= no (*go to next section*)

   a. If "YES," are you still taking estrogen? (1= yes   2= no) ☐

   b. Why do(did)you use estrogen ? (1 = yes   2 = no   9=unknown)

       i.   post surgery (hysterectomy and removal of ovaries) ☐

       ii.   relief of menopause symptoms ☐

       iii. prevent bone loss ☐

       iv.   protect against heart disease ☐

       v.   doctor's advice ☐

8. How old were you when you started using estrogen?   *Indicate the age in years.* ☐☐

9. How many years altogether did you take estrogen?   *Specify the duration in years.* ☐☐

ROSE QUESTIONNAIRE FOR ANGINA AND INTERMITTENT CLAUDICATION

ID number:

Section A: Chest Pain on Effort

1.   Have you ever had any pain or discomfort in your chest?
      1 = yes   2 = no (*go to Section C*)

2.   Do you get it when you walk uphill, upstairs or hurry?
      1 = Yes   2 = No (*go to Section B*)
      3 = Never hurries or walks uphill or upstairs

3.   Do you get it when you walk at an ordinary pace on the level?
      1 = Yes   2 = No

4.   What do you do if you get it while you are walking?
      1 = Stop or slow down   2 = Carry on (*go to Section B*)
      (*Record "stop or slow down" if subject carries on after taking nitroglycerine.*)

5.   If you stand still, what happens to it ?
      1 = Relieved   2 = Not relieved (*go to Section B.*)

6.   How soon ?
      1 = 10 minutes or less   2 = More than 10 minutes (*go to Section B.*)

7.   Will you show me where it was ? (*Record all areas mentioned. Use the diagram below to show the location if participant cannot tell exactly.*)
      1 = yes   2 = no
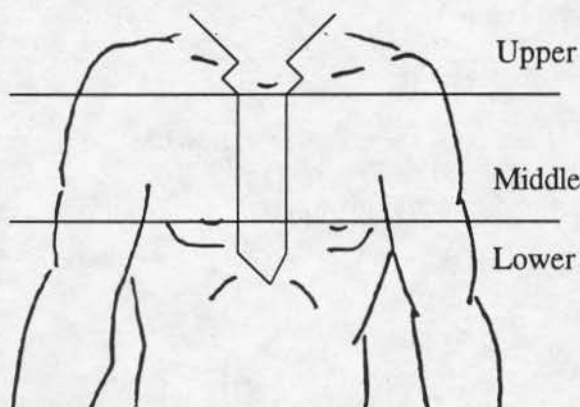
Sternum (upper or middle)

Sternum (lower)

Left anterior chest

Left arm

Other: _____

8.   Do you feel it anywhere else ?
      1 = Yes   2 = No
      If "YES," record additional information :_____

Section B: Possible Infarction

9. Have you ever had a severe pain across the front of your chest lasting for half an hour or more?
    1 = Yes   2 = No

☐

Section C: Intermittent Claudication

10. Do you get pain in either leg on walking ?
    1 = Yes   2 = No (*Go to Question 19*)

☐

11. Does this pain ever begin when you are standing still or sitting ?
    1 = Yes (*Go to Question 19*)   2 = No

☐

12. In what part of your leg did you feel it ?
    1 = Pain includes calf/calves
    2 = Pain does not include calf/calves (*Go to Question 19*)

    If calves not mentioned, ask: Anywhere else and specify: _____

☐

13. Do you get it if you walk uphill or hurry ?
    1 = Yes   2 = No (*Go to Question 19*)
    3 = Never hurries or walks uphill

☐

14. Do you get it if you walk at an ordinary pace on the level ?
    1 = Yes   2 = No

☐

15. Does the pain ever disappear while you are walking ?
    1 = Yes (*Go to Question 19*)   2 = No

☐

16. What do you do if you get it when you are walking ?
    1 = Stop or slow down   2 = Carry on (*Go to Question 19*)

☐

17. What happens to it if you stand still ?
    1 = Relieved   2 = Not Relieved (*go to Question 19*)

☐

18. How soon ?
    1 = 10 minutes or less   2 = More than 10 minutes

☐

*** END OF ROSE QUESTIONNAIRE ***

19. Code number of person completing this form

☐☐☐

20. Date of data collection

☐☐ / ☐☐ / ☐☐
mo   day   yr

Instructions for
Medical History Interview

Before beginning, make certain that the correct study identification number or the subject is entered at the top of the form. Explain to the subject some questions need to be asked about his or her medical history so that we can better evaluate whether or not he/she has heart disease or a tendency for heart disease. Stress that the information will be confidential and that their name will never be used in any data analysis.

A.   Current Medications

"It is important that we are able to identify all of the pills and medicines that you are now taking. We will talk about each one of the medicines that you brought with you. For each one, we would like to know whether you take it regularly. Don't worry if you forgot to take it when you were supposed to; just tell us as accurately as you can how often you have taken." The interviewer then proceeds to ask about each medicine that the patient brought with him/her, and records for each, the name on the bottle. If the bottle is unlabeled, record the color and shape of the pill and save one of them so that it can be identified in the PDR later. After you have gone through all of the medicines that the patient brought with him/her, then ask "Are there any medicines that you are taking that you forgot to bring", if the answer is yes, record them also in Section A. After the clinic is over, it will be necessary to review Section A, first to identify medicines which were not named, then, to enter a medication code for each of those medicines. Leave the NDC code and Class code blank. They will be coded later in the SHS coordinating center.

B.   We would appreciate it if you can give us information about your past medical history.

I am going to ask about a number of medical conditions. Did you ever see a doctor or other health care professional for any of the problems that I am going to mention. (Note to Interviewer: When inquiring about how many years ago, if the patient has trouble remembering, try to ask in what year or how old they were when they had the condition; we can then calculate from their current age or from the current year, the number of years ago and enter it in the appropriate box).

4.   High Blood Pressure. For high blood pressure, the interviewer should be alert for those individuals who answer no, who might in fact have been prescribed or taking medication for hypertension. If the patient does not know when the hypertension first began, ask when they first began taking medication for high blood pressure and record that date.

5.   Arthritis. The interviewer should also inquire about arthritis.

6.   Cancer. The interviewer, when inquiring about cancer should ask about cancer and

diseases such as leukemia, lymphoma and tumors of the skin. If they answer yes, record the type of cancer.

7.   Diabetes and type of treatment. The interviewer should be alert to individuals who reply no, who are in fact taking oral hypoglycemic agents or insulin.

8.   Kidney Failure. The interviewer should describe this as kidney failure or if he/she has been told that their kidneys are not working.

9-10.   Renal dialysis and transplantation. When inquiring about renal dialysis, the interviewer should also ask if the patient must go two or three times a week to have a machine cleanse their blood.

11.   Cirrhosis of the Liver or Yellow Jaundice. The interviewer should stress that this can occur both because of alcohol and for other reasons as well.

12.   Lung problems. When inquiring about emphysema, the interviewer should also ask about difficulty in breathing.

13.   Heart catheterization. Ask if patient had any kind of heart catheterization. If "yes," determine whether they had an angioplasty or other procedure, the date of the procedure and also the hospital where it was done. Using the Procedures and Tests Photocopy Check List, obtain the medical record of this procedure (the catheterization report and pictures) and the narrative hospital summary for review by the SHS Morbidity Review Panel. Be sure to have PARTICIPANTS sign the release forms for non-IHS HOSPITAL, if HOSPITALIZATIONS occurred since the Phase I examination. Attach the Photocopy Checklist to medical records materials and forward the packet to the Coordinating Center.

14.   Treadmill test or exercise test to examine the heart. If "yes," determine the date of the procedure and the hospital where it was done. Using the Procedures and Tests Photocopy Check List, obtain the medical record of this procedure (the ECG paper from the test and the report) and the narrative hospital summary for review by the SHS Morbidity Review Panel. If the test included use of thallium, also obtain a copy of the nuclear medicine report. Be sure to have PARTICIPANTS sign the release forms for non-IHS HOSPITAL, if HOSPITALIZATIONS occurred since the Phase I examination. Attach the Photocopy Checklist to medical records materials and forward the packet to the Coordinating Center.

15.   Whether the participant had taken any ECG since the last SHS exam?

16.   Heart failure. "That is, did the doctor or health care provider ever tell you that your heart was not working properly?" The necessity to sleep with several pillows (orthopnea) suggests heart failure.

17. Heart Attack. When inquiring about heart attack, this would usually have involved hospitalization, but in some instances, the patient could have been told they had a heart attack in the past on the basis of an electrocardiogram. If the patient indicates that he/she had a heart attack, ask if there were more than one. Obtain information about each hospitalization and record in (o) below so that medical records can be reviewed for morbid event. Be sure to have participants sign the release forms for non-IHS hospitals, if hospitalizations occurred in the last ten years.

18. If the patient indicates that he/she has had other heart trouble, the interviewer should ask about the symptoms, because of the possibility that it may in fact fit in a, b or c above. If any of these procedures were done since Phase I exam, use the Procedures and Tests Photocopy Check List and obtain a copy of the test or operative report and the narrative hospital summary for review by the SHS Morbidity Review Panel. Be sure to have PARTICIPANTS sign the release forms for non-IHS HOSPITAL, if HOSPITALIZATIONS occurred since the Phase I examination. Attach the Photocopy Checklist to medical records materials and forward the packet to the Coordinating Center.

19. Stroke. Ask if the patient indicates that he/she has not had a stroke, ask also whether he/she has had any episode where he/she suddenly could not move a part of his/her body for a prolonged period of time. Obtain information about each hospitalization and record in (Question 20) below so that medical records can be reviewed for morbid event. Be sure to have participants sign the release forms for non-IHS hospitals, if hospitalizations occurred in the last ten years.

20. Ask the participant whether he/she had been hospitalized for heart attack and/or stroke or other cardiovascular problems in the past ten years. Record the hospital names, town and state where the hospital is located, date of hospitalization and the reason of the hospitalization.

21-31. Respiratory questions. Ask the participants whether he/she had any of the respiratory problems in this section.

INSTRUCTIONS FOR REPRODUCTION AND HORMONE USE: WOMEN ONLY

If the patient is a female, explain that we know that in many cases, women appear to be protected from heart disease. Therefore it is necessary for us to ask some questions about their reproductive history, because we are trying to better understand why women appear to have less heart disease.

1. Ask participants whether she breast fed her last child for at least a month.

2. Ask participants whether she had been told that her blood sugar was high in any of the pregnancies.

3.	When inquiring about menstrual cycles stopping permanently, this means for more than one year.

4.	If the patient does not know how old she was when they stopped, ask if she remembers what year was the last year that she had a menstrual period. By referring to the patients' age and the year in which the periods stopped, then the interviewer can compute the age when they stopped completely.

5.	The interviewee should answer whether the menopause or the cessation of periods occurred naturally or whether it occurred after an operation to remove the womb or uterus.

6-9.	Use questionnaire as written. If patients are currently taking estrogen pills or birth control pills, be sure they are recorded on the medication history.

INSTRUCTIONS FOR USE OF THE ROSE QUESTIONNAIRE FOR ANGINA AND INTERMITTENT CLAUDICATION

This questionnaire, originally developed by Rose & Blackburn, has been the mainstay of cardiovascular disease surveys for a number of years. The primary feature of this questionnaire is to have a standardized assessment for the pain associated with angina and intermittent claudication. Since it is well recognized that there can be many other causes for both chest and leg pain, the main objective of the questionnaire is to ask a series of questions so that certain patterns of pain will be assigned positively and others will not be assigned. For this reason, it is important that the questions be asked in the order stated. In addition, during several points of the questionnaire, there is an asterisk if a certain answer is received. The purpose of this asterisk is to assure that the questioner then proceeds to the next section. If an answer is received that has an asterisk, it has been determined that this answer indicates that the pain is not characteristic of either angina or intermittent claudication and thus, it is not necessary to proceed with that section.

The questions are essentially self-explanatory. It is permissible, and in fact advisable, when referring to pain or discomfort in the chest to elaborate to describe this pain as a tightening or crushing feeling that may or may not radiate onto the left arm.

In addition, since this is a standardized questionnaire developed in Britain, phrases such as "carry-on" can also be described as "keep on going" or "continue to walk or climb".

THE STRONG HEART STUDY PHASE II

PHYSICAL EXAMINATION

ID number:

Social Security Number:

Before examinations start, check TOBACCO AND CAFFEINE USE

"Tobacco, alcohol, caffeine and activity levels can change the results of the exams and laboratory tests we will do today. Because of this, we will ask you a few questions."

1. Have you smoked or used chewing tobacco or snuff within the last 4 hours ?
    1= yes   2= no (*Skip to Question 2*)

    a. How long ago did you last smoke or last use chewing tobacco or snuff?
        Specify the lag by hours.

    b. If less than an hour, specify the minutes.

2. Did you consume more than 5 alcoholic drinks in the past 24 hours? (1=Yes, 2=No)

3. Did you perform vigorous physical activity in the past 24 hours? (1=Yes, 2=No)

"We are going to ask you not to smoke or use chewing tobacco until you have completed your visit with us today. We do this so that your test results are not affected by tobacco use. If you must use tobacco, please tell us that you did before you leave."

4. Have you had any coffee, tea, caffeinated soft drink or chocolate within the last 4 hours ?
    1=yes   2=no (*Skip to Section I*)

    a. How long ago did you last have any coffee, tea, caffeinated soft drink or
        chocolate? Specify the lag by hours

    b. If less than an hour, specify the minutes

I. **STANDING MEASUREMENT:** With shoes removed, heavy articles from pockets removed, and participant standing, measurements should not be made over gown or scub suit. Fractions less than 0.5 will be omitted, and fractions greater than or equal to 0.5 will be rounded up to the next higher unit.

5. Height in CENTIMETERS (cm)   (Optional: _____ inches)

6. Weight in KILOGRAMS(kg)   (Optional: _____ pounds)

7. Hip circumference in CENTIMETERS (cm) (Optional: _____ inches)

II.   SITTING MEASUREMENT

8.   Right arm circumference, measured in centimeters (cm)
      Midway between acromium and olecranon   ☐☐

9.   Cuff size (arm circumference in brackets)   ☐
      1= Pediatric (under 24cm)   3= Large arm (33-41cm)
      2= Regular arm (24-32cm)   4= Thigh (>41cm)

10.   Pulse obliteration pressure   ☐☐☐

A.   FIRST BLOOD PRESSURE MEASUREMENT
      (After 5 minutes in sitting position - right arm)

11.   Systolic, Phase I - first sound   ☐☐☐

12.   Diastolic, Phase V - first silence in a series of at least two silences ☐☐☐
      (If Phase V did not appear, record Phase IV)

B.   SECOND BLOOD PRESSURE MEASUREMENT *(after raising the arm for 5 seconds
      and resting it on the table for another 25 seconds)*

13.   Systolic, Phase I - first sound   ☐☐☐

14.   Diastolic, Phase V - first silence in a series of at least two silences ☐☐☐
      (If Phase V did not appear, record Phase IV)

C.   THIRD BLOOD PRESSURE MEASUREMENT *(after raising the arm for 5 seconds and
      resting it on the table for another 25 seconds)*

15.   Systolic, Phase I - first sound   ☐☐☐

16.   Diastolic, Phase V - first silence in a series of at least two silences ☐☐☐
      (If Phase V did not appear, record Phase IV)

17.   Were the above blood pressures taken from LEFT arm because of missing right arm ☐
      or some other reason ?
      1=yes, If yes, specify:_____
      2=no

18.   Recorder ID (For the SHS staff who took BPs):   ☐☐☐

19.   Time of day *(Please use military time, hour:minute)*   ☐☐ : ☐☐

D. EXAMINATION OF THE CHEST

20. Examination of the lungs (Use the following codes to fill in the table)
    1=clear   3=rhonchi
    2=rales   4=both

LOCATION	Right Posterior Lung	Left Posterior Lung
Apex		
Mid		
Lower		

E. EXAMINATION OF NECK VEINS, BRUITS (CAROTID)

21. a. Left   (1=distended   2=flat)   ☐

    b. Right (1=distended   2=flat)   ☐

22. a. Right carotid bruit (1=present   2=absent)   ☐

    b. Left carotid bruit (1=present   2=absent)   ☐

III. SUPINE MEASUREMENTS

23. Right femoral bruit: 1=Present   2=Absent   3=Missing limbs   ☐

24. Left femoral bruit: 1=Present   2=Absent   3=Missing limbs   ☐

25. Waist measurement at umbilicus, in CENTIMETERS (cm)   ☐☐☐
    (Optional: _____inches)

26. Evidence of chest surgery or chest deformity ?   ☐
    1=Yes   2=No (*Skip to Section A*)

a. If "Yes," ask: "Did you have lung surgery?"   ☐
    1=Yes   2=No (*Skip to b*)

    If "Yes," when and where?   ☐☐/☐☐/☐☐
                                                     mo   day   yr

    hospital/clinic: _____

    If "Yes," what type of surgery?   ☐
    1=Lobe of lung removed
    2=Entire lung removed
    3=other, specify: _____

b. Did you have heart surgery?
    1=Yes   2=No (*skip to Section A*)

    □

    If "Yes," which surgery have you had?

    i.   Bypass?   1=Yes   2=No

    □

    If "Yes," when and where?
    (*Record the most recent*)

    □□ / □□ / □□
    mo   day   yr

    hospital/clinic _____

    ii.   Valvular repair/replacement? 1=Yes   2=No

    □

    If "Yes," when and where?
    (*Record the most recent*)

    □□ / □□ / □□
    mo   day   yr

    hospital/clinic _____

    iii. Pacemaker?   1=Yes   2=No

    □

    If "Yes," when and where?
    (*Record the most recent*)

    □□ / □□ / □□
    mo   day   yr

    hospital/clinic _____

    iv.   Other?   1=Yes   2=No

    □

    Specify: _____

    If "Yes," when and where?
    (*Record the most recent*)

    □□ / □□ / □□
    mo   day   yr

    hospital/clinic _____

A. ECG AND IMPEDANCE MEASUREMENT

27. Electrocardiogram reading (*preliminary reading from ECG machine*)
    1= Normal   4= Otherwise normal
    2= Abnormal   9= Unclassified
    3= Borderline

    □

28. Impedance measurement

    a. Resistance

    □□□

    b. Reactance

    □□

    c. Taken on left side because of amputation?   (1=yes, 2=no)

    □

    d. Not taken because of amputation   (1=yes, 2=no)

    □

B.   PEDAL PULSES AND EDEMA

For the following items (29 to 32), use the following codes for findings:
    1=present,   2 = absent,   3 = missing limbs.

29.   Right posterior tibial pulse   ☐

30.   Right dorsalis pedis pulse   ☐

31.   Left posterior tibial pulse   ☐

32.   Left dorsalis pedis pulse   ☐

33.   Pedal edema   ☐
        (1=absent, 2=mild, 3=marked, above midpoint between malleolus and patella)

C.   DOPPLER BLOOD PRESSURE

Doppler blood pressure is measured in the posterior tibial artery. If not audible, use dorsalis pedis.
If neither is audible, record zero. Record 999 if participant refuses or if blood pressure is not taken
for a medical reason or amputation. Record 888 if you cannot obliterate.

34.   Right arm Doppler blood pressure - brachial artery
        Use left arm if left arm was used for standard blood pressure reading.

        a)   First systolic B.P. measurement   ☐☐☐

        b)   Second systolic B.P. measurement (*no waiting time needed*)   ☐☐☐

35.   Right ankle Doppler blood pressure

        a)   First systolic B.P. measurement   ☐☐☐

        b)   Second systolic B.P. measurement (*no waiting time needed*)   ☐☐☐

        c)   Location: 1=posterior tibial   2=dorsalis pedis   ☐

36.   Left ankle Doppler blood pressure

        a)   First systolic B.P. measurement   ☐☐☐

        b)   Second systolic B.P. measurement (no waiting time needed)   ☐☐☐

        c)   Location: 1=posterior tibial   2=dorsalis pedis   ☐

D.   EXAMINATION OF EXTREMITIES FOR AMPUTATIONS

37.   Are any extremities missing ?   ☐
      1=yes, *fill out the questions in the following table.*
      2=no,   *skip to SECTION IV.*

      If "YES" to amputation , Code the cause of amputation:
            1 = Diabetes   4 = Other, please specify
            2 = Trauma   9 = Unknown
            3 = Congenital

Extremities	Yes / No	Cause	Comment
a. Right arm			
b. Right hand			
c. Right finger(s)			
How many:			☐
d. Left arm			
e. Left hand			
f. Left fingers			
How many:			☐
g. Right leg above knee			
h. Right leg below knee			
i. Right foot/toes			
How many:			☐
j. Left leg above knee			
k. Left leg below knee			
l. Left foot/toe			
How many:			☐

IV.   ADMINISTRATIVE INFORMATION

38.   Code number of person completing this form   ☐☐☐

39.   Date of data collection   ☐☐ / ☐☐ / ☐☐
                                                   mo   day   yr

GTT CHECKLIST

ID number: ☐☐☐☐☐☐

Center: _____   Today's Date (mm/dd/yy): ☐☐/☐☐/☐☐

1. Fasting One Touch glucose result. *If not done, draw two lines across the boxes.* ☐☐☐

2. Is blood sample taken?   ☐
        1 = yes, and participant has been fasting,
        2 = yes, but participant has NOT been fasting,
        3 = no, participant is on renal dialysis,
        4 = no, participant has had a kidney transplant,
        5 = no, participant has not been fasting,
        6 = participant refused,
        7 = other, specify: _____

    If blood sample is NOT taken because of dialysis/transplantation or refusal,   ☐
    are tubes of blood for DNA and RBC typing taken?
    1=yes   2=no

3. When was the last time you ate   _____   AM   PM

4. Time of collection of fasting samples   _____

5. Time the 75 gram glucose beverage was consumed   _____

6. Time of collection of urine sample   _____

7. Time of 2-hr blood sample   _____

8. **The participant did not have GTT because of:** *Check the appropriate answer(s)*

    a.   diabetes, on insulin treatment   ☐

    b.   diabetes, on oral agent   ☐

    c.   One Touch > 225 mg/dl   ☐

    d.   refusal to have GTT done   ☐

9. Has the participant vomited after the glucose beverage was given?   ☐
    (1=yes   2=no)

    If yes, when? (Indicate the time) _____

Comments : _____
_____

Standing and Sitting Measurement

ID number:

Social Security Number:

I. **STANDING MEASUREMENT:** With shoes removed, heavy articles from pockets removed, and participant standing, measurements should not be made over gown or scub suit. Fractions less than 0.5 will be omitted, and fractions greater than or equal to 0.5 will be rounded up to the next higher unit.

1. Height in CENTIMETERS (cm)

2. Weight in KILOGRAMS (kg)

3. Hip circumference, in CENTIMETERS (cm)

II. SITTING MEASUREMENT

4. Right arm circumference, measured in CENTIMETERS (cm)
   Midway between acromium and olecranon

5. Cuff size (arm circumference in brackets)
   1= Pediatric (under 24cm)   3= Large arm (33-41cm)
   2= Regular arm (24-32cm)   4= Thigh (>41cm)

6. Pulse obliteration pressure

A. FIRST BLOOD PRESSURE MEASUREMENT
   (*After 5 minutes in sitting position - right arm*)

7. Systolic, Phase I - first sound

8. Diastolic, Phase V - first silence in a series of at least two silences
   (*If Phase V did not appear, record Phase IV*)

B. SECOND BLOOD PRESSURE MEASUREMENT (*after raising the arm for 5 seconds and resting it on the table for another 25 seconds*)

9. Systolic, Phase I - first sound

10. Diastolic, Phase V - first silence in a series of at least two silences
    (*If Phase V did not appear, record Phase IV*)

C. THIRD BLOOD PRESSURE MEASUREMENT *(after raising the arm for 5 seconds and resting it on the table for another 25 seconds)*

11. Systolic, Phase I - first sound ☐☐☐

12. Diastolic, Phase V - first silence in a series of at least two silences ☐☐☐
(If Phase V did not appear, record Phase IV)

13. Were the above blood pressures taken from LEFT arm because of missing right arm or some other reason ? ☐
1=yes, If yes, specify _____
2=no

14. Recorder ID: ☐☐☐

15. Time of day *(Please use military time, hour:minute)* ☐☐ : ☐☐

16. Date of data collection ☐☐ / ☐☐ / ☐☐
                             mo   day   yr

APPENDIX 7 (b)
THE STRONG HEART STUDY PHASE II
PHYSICAL EXAMINATION -- QC DUPLICATE MEASUREMENT
Supine Measurement

ID number:

Social Security Number:

I.   SUPINE MEASUREMENT

1.   Waist measurement at umbilicus, in centimeters (cm)

2.   Impedance measurement

    a. Resistance

    b. Reactance

    c. Taken on left side because of amputation?   (1=yes,   2=no)

    d. Not taken because of amputation   (1=yes,   2=no)

DOPPLER BLOOD PRESSURE

Doppler blood pressure is measured in the posterior tibial artery. If not audible, use dorsalis pedis. If neither is audible, record zero. Record 999 if participant refuses or if blood pressure is not taken for a medical reason or amputation. Record 888 if you cannot obliterate.

3.   Right ankle Doppler blood pressure

    a)   First systolic B.P. measurement

    b)   Second systolic B.P. measurement (*no waiting time needed*)

    c)   Location: 1=posterior tibial   2=dorsalis pedis

4.   Left ankle Doppler blood pressure

    a)   First systolic B.P. measurement

    b)   Second systolic B.P. measurement (*no waiting time needed*)

    c)   Location: 1=posterior tibial   2=dorsalis pedis

For item 5, use left arm if left arm is used for standard blood pressure reading.

5. Right arm Doppler blood pressure - brachial artery

    a)   First systolic B.P. measurement   ☐☐☐

    b)   Second systolic B.P. measurement (*no waiting time needed*)   ☐☐☐

II. ADMINISTRATIVE INFORMATION

6. Code number of person completing this form   ☐☐☐

7. Date of data collection   ☐☐ ☐☐ ☐☐
                                                   mo   day   yr

APPENDIX 8

Checklist for Quarterly Observation of BP Technicians and New Employees by BP Supervisor (To be sent quarterly to the Coordinating Center)

BP Technician Code # _____   Observer Code # _____

Date Observed   /   /   (Month/Day/Year)

===

Instructions: For each item, check "yes" or "no" in the space provided to indicate if the procedure is carried out correctly. Record any comments in the blank line between that item and the next. For certain items specific parts of the procedure which are important are listed separately.

Measures arm for correct cuff size	( ) Yes	( ) No
Palpates brachial artery	( ) Yes	( ) No
Marks pulse point	( ) Yes	( ) No
Wraps cuff center of bladder over brachial pulse	( ) Yes	( ) No
Instructs on Posture	( ) Yes	( ) No
Full five minutes for rest allowed	( ) Yes	( ) No
Work station free of excessive noise	( ) Yes	( ) No
Finds Pulse obliteration point using standard manometer	( ) Yes	( ) No
Calculates peak inflation, standard manometer	( ) Yes	( ) No
Places stethoscope in ears	( ) Yes	( ) No
Inflates rapidly to peak	( ) Yes	( ) No
Counts full 5 seconds with pressure steady	( ) Yes	( ) No
Places bell on brachial pulse	( ) Yes	( ) No
Deflates cuff 2 mmHg per second	( ) Yes	( ) No
Deflates cuff after 2 absent sounds	( ) Yes	( ) No
Records readings	( ) Yes	( ) No
Disconnects tubes	( ) Yes	( ) No
Instructs to hold arm vertical for full 5 seconds	( ) Yes	( ) No
Waits at least 30 seconds before proceeding	( ) Yes	( ) No
Informs participant of average readings of 2nd and 3rd blood pressure	( ) Yes	( ) No

Special Comments:

APPENDIX 9

Form for Recording Simultaneous Blood Pressure Observations On A Volunteer by Two Technicians

Instructions: Approximately every 4 months, each technician should be part of a pair of technicians who simultaneously measure blood pressure using a Y-tube on a volunteer (not a Strong Heart participant). Each technician should separately record his/her measurements on a standard paper Strong Heart SBP form. The blood pressure supervisor should then transfer the results to this form and calculate the differences between the two sets of measurements. If the difference on any individual measurement is greater than 4 mmHg, or if the averages of the three readings for each technician differ by more than 3 mmHg, the supervisor should indicate the corrective action taken on this form. Any further sets of simultaneous measurements for a given pair should appear on a new form. A copy of each form should be sent to the Coordinating Center.

[handwritten margin note: Bold & underline]

Technician IDs:	1st ID: 1st Technician	2nd ID: 2nd Technician	Date: Difference
a. Initial Arm Circumference (cm)			
b. Initial Cuff Size Selected			
c. Pulse Obliteration Pressure			
d. First SBP			
e. First DBP			
f. Second SBP			
g. Second DBP			
h. Third SBP			
i. Third DBP			
j. Average SBP			
k. Average DBP			

Action taken if differences between technicians exceed limits specified:

APPENDIX 10

Monthly Log for Sitting Blood Pressure Station

Field Center: Arizona _____ Oklahoma _____

Pine Ridge _____   Eagle Butte _____   Ft. Totten _____

Month _____ Year _____

Monthly Check Procedures:

		Month				
		1	2	3	4	5
1.	Sphygmomanometer:	=========================
	Date of Check	—	—	—	—	—
	A. Check Tube for Oxide Dust	—	—	—	—	—
	B. Check Cap for Tightness	—	—	—	—	—
	C. Check that mercury is at zero with no pressure	—	—	—	—	—

List any problems found and corrective action taken:

Procedures performed only if there appear to be problems:

D.   If mercury bounces even though the cap appears tight, remove cap, clean of any mercury beads, and check opening at top of tube for dust

       Check Needed and Performed during weeks 1   2   3   4   5
       (Circle number of weeks applicable)

E.   If tube appears "dirty" (oxidized mercury) remove cap, tip manometer to retract mercury, run pipe cleaner down, replace cap

       Needed and Performed during weeks   1   2   3   4   5

F.   For any other problems contact control center for instructions before proceeding. List the problem encountered, the date, and the actions taken below:

2.   Measuring tape for arm circumference worn or stretched Check by holding the zero mark of the tape against the ruler used to measure standing height at the 150 cm mark. If the 30 cm mark on the tape used for arm circumference falls outside the range 119.5 to 120.5 on the standing height ruler it should be replaced.

Month:	1	2	3	4	5

===

Date of check: _____ _____ _____ _____ _____

Point on height
ruler where 30
cm on tape falls   _____ _____ _____ _____ _____

APPENDIX 11

Maintenance Procedures for Standard Sphygmomanometer

The following checks should be conducted at least every month, and a log kept of the dates and the people carrying out the troubleshooting (see Appendix IV).

1. With the instrument placed flat on the table, and the inflation system disconnected, the level of mercury should read zero in the standard instrument. If the reading is either above or below the zero mark, mercury should be added or withdrawn until it does read zero. The top of the meniscus is on the zero line when the eyes are level with this line and the mercury is correctly adjusted.

2. The inflation system should then be reconnected, and the cuff rolled around a bottle and secured. The valve should be closed on the Air Flo system, and the instrument inflated until the mercury rises to 240 mmHg. The Air Flo valve should then be slowly opened and the mercury allowed to fall to 200 mmHg. The valve should then be closed, at which time the mercury column should remain stable. If the column continues to fall, there is an air leak, and the following steps should be taken:

3. The system should be reinflated until the column rises to 200 mmHg. The tubing should be pinched at various locations to localize the area of the leak. Appropriate replacement of the tubing, cuff, or valve should be performed.

4. With the instrument inflated above full calibration, the screw cap should be examined for mercury leaks. If this happens, the screw cap should be tightened. If the leak persists or the mercury is seen at the bottom of the tube, the silicone rubber which provides a seat for both ends of the glass tube should be replaced.

5. With time, the mercury will become dirty and an oxide layer will be deposited on the inside of the glass tube. The instrument should be laid nearly on its side (on a tray) so   that the mercury will return to the reservoir and none can be seen in the glass tube. The tube should be removed carefully and cleaned out using the long pipe cleaner supplied with this instrument. The tube should then be replaced and the zero level rechecked.

    Since mercury is a toxic substance, all maintenance procedures must be performed carefully and with attention to safety. Mercury should not be allowed to get in contact with rings and other jewelry.

APPENDIX 12 -- MAC-PC Setup

STOP
RETURNS ECG CART TO
MAIN MENU. TERMINATES
PRINTING OF A REPORT.

DESTRUCTIVE BACKSPACE
DELETES ALPHANUMERIC
CHARACTER IMMEDIATELY
TO THE LEFT OF CURSOR.

RECORD RHYTHM
PRINTS A 3-LEAD OR
6-LEAD RHYTHM REPORT.

FUNCTION KEYS
SELECTS FUNCTION FROM
LCD DISPLAY THAT IS
DIRECTLY ABOVE KEY OR
ALTERNATE FUNCTION (↑) KEY. *

LCD DISPLAY
PRESENTS EACH PROMPT
OR MENU FOR ECG TEST.

RECORD ECG
PRINT A
12-LEAD REPORT.

**SHIFT/ALTERNATE
FUNCTION KEY**
CHANGES TO
CHARACTER
DISPLAYED ON
TOP OF KEY
OR ALTERNATE
FUNCTION (↑)
KEY.

SPACE BAR

**POWER
SWITCH**

RIGHT ARROW
MOVES CURSOR RIGHT
ONE SPACE AT A TIME.

ENTER
COMPLETES INPUT-
TELLS SYSTEM TO
GO TO NEXT DISPLAY.

LEFT ARROW
MOVES CURSOR LEFT
ONE SPACE AT A TIME.

SHIFTED CONTRAST KEYS
SHIFTED DOWN ARROW
PRESSED SIMULTANEOUSLY
WITH THE SHIFT KEY. LIGHTENS
THE LCD DISPLAY. SHIFTED UP
ARROW PRESSED SIMULTANEOUSLY
WITH THE SHIFT KEY, DARKENS
THE LCD DISPLAY.

UP ARROW
RETURNS LCD DISPLAY
TO PREVIOUS PROMPT
OR MENU.

* FOR MOST FUNCTION KEY USES, PRESSING EITHER THE NORMAL OR THE ALTERNATE FUNCTION (↑) KEY PRODUCES THE
  SAME RESULTS.

APPENDIX 12 (a)   Cardiograph Setup

Although your MAC PC will operate perfectly when you first receive it from the factory, you'll want to 'set up' a lot of the details such as date and time, the name of your institution, types of reports you want printed, etc. Once these details are set, the cardiograph will retain them until you change the details again.

To turn on Power , press   `•/d`

To begin cardiograph setup, press   ⊘   to display the *Main Menu:*

```
↑Task     V1 + II + V5
PatInfo         Rhythm          25mm/s         10mm/mV   100Hz
```

Next, press   ◇   and   `F1↑ 2`   at the same time to display the System Functions

menu:

```
                        System Functions
      Storage         Setup    Diag        RevXmit        Monitor
```

`F2 2` `F2↑ 4`

Select Setup (F2) by pressing either   `F2 3`   or   `F2↑ 4`   . The following display will appear if
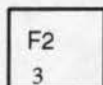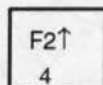
a Level 1 password has been entered:

```
Password:_____
```

Press keys "L" and "1" (numeric one, not lowercase "l"), then press   ⬅⏎   to display the

first Cart Setup menu:

Cart Setup

F1 1	F1↑ 2	F2 3	F2↑ 4	F3 5	F3↑ 6	F4 7	F4↑ 8	F5 9	F5↑ 0

Dat/Tim   Phone   LdGrps   Reports   More

Step A   **Step B**   **Step C**   **Step D**

Cart Setup

Modem   Passwds   Misc   Defaults   More

F1 1	F1↑ 2	F2 3	F2↑ 4	F3 5	F3↑ 6	F4 7	F4↑ 8	F5 9	F5↑ 0

Step E   **Step F**   **Step G**   **Step H**
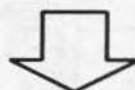
Cart Setup

Timeout   RS232   Monitor More

F1 1	F1↑ 2	F2 3	F2↑ 4	F3 5	F3↑ 6

Step I   **Step J**   **Step K**

Each of the above steps is explained in the following pages.

<u>Step</u>	<u>Contents</u>
A.	Date/Time
B.	Phone
C.	Lead Groups
D.	Report Formats
E.	Auto Dial
F.	Passwords
G	Miscellaneous
H.	Defaults
I.	Timeout

Step A: Date and Time Setup

Date and Time Setup	Press Backspace-Delete to erase.
Date Time	

```
┌────┐  ┌────┐
│ F1 │  │F1↑ │
│ 1  │  │ 2  │
└────┘  └────┘
```

Today's Date (DD-MM-YY):	Type day + dash + month + dash
DD=Day, MM=Month Name, YY=Year	+year and press ⟵

Date and Time Setup	
Date Time	

```
    ┌────┐  ┌────┐
    │ F2 │  │F1↑ │
    │ 1  │  │ 2  │
    └────┘  └────┘
```

Time (HH-MM):	Type hour + dash + minute and
HH=Hour, MM=Minute (24 Hr Clock)	press ⟵

Date and Time Setup	Press ⊘ to return to
Date Time	the *Main Menu*.

Step B: Phone Setup

```
                         Cart Setup
Dat/Tim   Phone   LdGrps   Reports   More
```

```
┌──────┐  ┌──────┐
│  F2  │  │ F2↑  │
│  3   │  │  4   │
└──────┘  └──────┘
```

Some universities need 8 to get off campus, you need 9 or nothing. "=" gives a pause for off-campus dial tone. "1" is for long distance. The rest is the Fitzsimons EKG Center toll free access number.
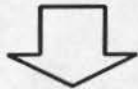
```
Phone Number
0 - 9 # * = ,                    9=18005314784
```

Type phone number. Then press

```
┌──────┐
│  ⏎   │
└──────┘
```

```
                         Cart Setup
Modem   Passwds   Misc   Defaults   More
```

Press ⬣ to return to the *Main Menu.*

Step C: Lead Groups-Rhythm Leads Setup

```
                    Lead Groups
Rhythm   Standrd   RMR   4 x 2.5
```

```
┌──────┐  ┌──────┐
│  F1  │  │ F1↑  │
│  1   │  │  2   │
└──────┘  └──────┘
```

These should never need to be changed.

```
Group:
AutoRhym   Group1   Group2   Group3   Group4
```

```
┌────┐ ┌────┐ ┌────┐ ┌────┐ ┌────┐ ┌────┐ ┌────┐ ┌────┐
│ F1 │ │F1↑ │ │ F2 │ │F2↑ │ │ F3 │ │F3↑ │ │ F4 │ │F4↑ │
│ 1  │ │ 2  │ │ 3  │ │ 4  │ │ 5  │ │ 6  │ │ 7  │ │ 8  │
└────┘ └────┘ └────┘ └────┘ └────┘ └────┘ └────┘ └────┘
```

```
┌────┐ ┌────┐
│ F5 │ │F5↑ │
│ 9  │ │ 0  │
└────┘ └────┘
```

Select a group. The previously chosen leads will appear. Then press

```
┌──────┐
│  ⏎   │
└──────┘
```

```
┌─────────────────────────────────────────────┐
│ Number of Rhythm Leads :      3              │
│            3       6                         │
└─────────────────────────────────────────────┘
```

Select the number of rhythm leads you want on writer reports. Then press ⏎

```
┌──────┐ ┌──────┐ ┌──────┐ ┌──────┐
│ F1   │ │ F1↑  │ │ F2   │ │ F2↑  │
│ 1    │ │ 2    │ │ 3    │ │ 4    │
└──────┘ └──────┘ └──────┘ └──────┘
```

Select 1 of 12 available leads for each of the 3 or 6 rhythm channels; press ⏎ after each selection. In the example below, the displays for the 12 available leads are shown for channel 1:

```
┌─────────────────────────────────────────────┐
│ Ch 1:  V1                                    │
│         I      II     III       More         │
└─────────────────────────────────────────────┘  ⏎
```

```
┌─────────────────────────────────────────────┐
│ Ch 2:  II                                    │
│         I      II     III       More         │
└─────────────────────────────────────────────┘  ⏎
```

```
┌─────────────────────────────────────────────┐
│ Ch 3:  V5                                    │
│         I      II     III       More         │
└─────────────────────────────────────────────┘  ⏎
```

After selecting a lead for each of the channels, the following will appear:

```
┌─────────────────────────────────────────────┐
│               Lead Groups                    │
│ Rhythm    Standrd     RMR         4 x 2.5    │
└─────────────────────────────────────────────┘
```

Press ⬡ to return to the *Main Menu*.

Step D: Report Formats Setup

```
┌─────────────────────────────────────────────┐
│            Report Formats for:               │
│ Confrmd    Unconf                            │
└─────────────────────────────────────────────┘
```

Do not configure confirmed.
Press F2 for unconfirmed.

```
┌──────┐ ┌──────┐ ┌──────┐ ┌──────┐
│ F1   │ │ F1↑  │ │ F2   │ │ F2↑  │
│ 1    │ │ 2    │ │ 3    │ │ 4    │
└──────┘ └──────┘ └──────┘ └──────┘
```
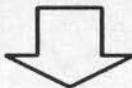
For each of the following LCDs press either F1 keys for "YES", F2 keys for "NO"; and press ⏎ to store the report information.

| Suppress Orig Rpt Interpretation: | NO |
| Yes | No |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

Clinic choice here. Marquette interpretation may be printed on ECG.

⬇

| Suppress Copy Interpretation: | NO |
| Yes | No |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

Phoenix enters F1 or F2 for "YES".

⬇

| Suppress Text Page: | NO |
| Yes | No |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

⬇

| Rhythm and Morphology Report (RMR): NO |
| Yes | No |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

⬇

(1)

| 1 Complex / Lead: | NO |
| Yes | No |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

⬇

(2)
Automatic Rhythm (1x10):			NO
Yes		No	

F1	F1↑	F2	F2↑
1	2	3	4
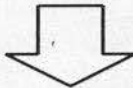
(3)
12 Lead (4x2.5):			YES
Yes		No	

This is the <u>ONLY</u> format to be printed.

F1	F1↑	F2	F2↑
1	2	3	4
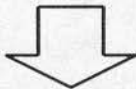
(4)
Separate Text Page for 4x2.5:		NO
Yes	No	

F1	F1↑	F2	F2↑
1	2	3	4
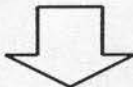
(5)
1 Page 4x2.5 with Rhythm:		NO
Yes	No	

F1	F1↑	F2	F2↑
1	2	3	4
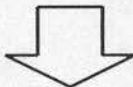
12 Lead (2x5):		NO
Yes	No	

F1	F1↑	F2	F2↑
1	2	3	4

12 Lead (2x10):		NO
Yes	No	

F1	F1↑	F2	F2↑
1	2	3	4

```
┌─────────────────────────────────────────────────────────────────────┐
│ 12 Lead  (4x10):                                    NO                │
│          Yes          No                                              │
└─────────────────────────────────────────────────────────────────────┘
┌──────┐ ┌──────┐ ┌──────┐ ┌──────┐
│ F1   │ │ F1↑  │ │ F2   │ │ F2↑  │
│ 1    │ │ 2    │ │ 3    │ │ 4    │
└──────┘ └──────┘ └──────┘ └──────┘

┌─────────────────────────────────────────────────────────────────────┐
│ 12 Lead (2x5 at 50mm/s):                            NO                │
│          Yes          No                                              │
└─────────────────────────────────────────────────────────────────────┘
┌──────┐ ┌──────┐ ┌──────┐ ┌──────┐
│ F1   │ │ F1↑  │ │ F2   │ │ F2↑  │
│ 1    │ │ 2    │ │ 3    │ │ 4    │
└──────┘ └──────┘ └──────┘ └──────┘

┌─────────────────────────────────────────────────────────────────────┐
│                  Report Formats for:                                  │
│ Confrmd          Unconf                                               │
└─────────────────────────────────────────────────────────────────────┘
```

From here, Press "RETURN" key.

When you return to the start, press ⊘ to return to the *Main Menu.*

Step E: Modem Setup -- Auto Dial

```
┌─────────────────────────────────────────────────────────────────────┐
│                  Cart Setup                                           │
│ Modem            Passwds        Misc    Defaults      More            │
└─────────────────────────────────────────────────────────────────────┘
┌──────┐ ┌──────┐
│ F1   │ │ F1↑  │
│ 1    │ │ 2    │
└──────┘ └──────┘
```

⬇
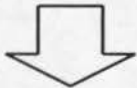
```
┌─────────────────────────────────────────────────────────────────────┐
│ Speaker On:    Dialing Only                                           │
│        Dial    Always                                                 │
└─────────────────────────────────────────────────────────────────────┘
┌──────┐       ┌──────┐
│ F1   │  or   │ F2   │
│ 1    │       │ 3    │
└──────┘       └──────┘

┌─────────────────────────────────────────────────────────────────────┐
│ Dialing: Auto Dial                                                    │
│          Manual        Auto                                           │
└─────────────────────────────────────────────────────────────────────┘
        ┌──────┐ ┌──────┐
        │ F2   │ │ F2↑  │
        │ 3    │ │ 4    │
        └──────┘ └──────┘

┌─────────────────────────────────────────────────────────────────────┐
│ Dialing Format: Touch Tone                                            │
│          Pulse            T Tone                                      │
└─────────────────────────────────────────────────────────────────────┘
┌──────┐       ┌──────┐
│ F1   │  or   │ F2   │
│ 1    │       │ 3    │
└──────┘       └──────┘
```

Or "PULSE", may vary by site.

```
┌─────────────────────────────────────────────────────────┐
│ Dial Tone Required:    YES                                │
│        Yes             No                                 │
└─────────────────────────────────────────────────────────┘
```

F1 1	or	F2↑ 4

```
┌─────────────────────────────────────────────────────────┐
│ Dial Tone Time: 1s                                        │
│        1s              2s                                 │
└─────────────────────────────────────────────────────────┘
```

F1 1	or	F2↑ 4

```
┌─────────────────────────────────────────────────────────┐
│ Modem Transmit Power Level:   -9dBm                       │
│ -6dBm   -7dBm  -8dbM  -9dBm     More                      │
└─────────────────────────────────────────────────────────┘
```

F1 1	F1↑ 2	F2 3	F2↑ 4	F3 5	F3↑ 6	F4 7	F4↑ 8	F5 9	F5↑ 0

```
┌─────────────────────────────────────────────────────────┐
│ Transmit Synch Time:   148.3 ms                           │
│ 800ms          220ms          148.3ms       90ms    More  │
└─────────────────────────────────────────────────────────┘
```

F1 1	F1↑ 2	F2 3	F2↑ 4	F3 5	F3↑ 6	F4 7	F4↑ 8	F5 9	F5↑ 0

```
┌─────────────────────────────────────────────────────────┐
│ Answer Tone Frequency:     2025 Hz                        │
│ 2025hz         2100Hz                                     │
└─────────────────────────────────────────────────────────┘
```

F1 1	F1↑ 2	F2 3	F2↑ 4

```
┌─────────────────────────────────────────────────────────┐
│ Answer Tone Wait (in seconds): 180                        │
│ 5 - 600                                                   │
└─────────────────────────────────────────────────────────┘
```

Step F: Password Setup

```
┌─────────────────────────────────────────────────────────┐
│               Cart Setup                                  │
│ Modem         Passwds      Misc   Defaults     More       │
└─────────────────────────────────────────────────────────┘
```

Passwords are preset as
L1 for Level 1 as all
aspects of programmability.

F2 3	F2↑ 4

```
┌─────────────────────────────────────────────────────────┐
│               System Passwords                            │
│ Level 1       Level 2                                     │
└─────────────────────────────────────────────────────────┘
```

F1 1	F1↑ 2	F2 3	F2↑ 4

Step G: Miscellaneous Setup

```
┌─────────────────────────────────────────────────────────────────┐
│              Cart Setup                                           │
│  Modem      Passwds       Misc    Defaults      More              │
└─────────────────────────────────────────────────────────────────┘
                    ┌──────┐ ┌──────┐
                    │ F2   │ │ F2↑  │
                    │  3   │ │  4   │
                    └──────┘ └──────┘
```

For each of the following prompts, type in an appropriate response or press a function (F) key. Then

press [⟵] to store that information.

```
┌─────────────────────────────────────────────────────────────────┐
│  Line Frequency:                    60 Hz                         │
│  60 Hz            50 Hz                                           │
└─────────────────────────────────────────────────────────────────┘
┌──────┐ ┌──────┐ ┌──────┐ ┌──────┐
│ F1   │ │ F1↑  │ │ F2   │ │ F2↑  │
│  1   │ │  2   │ │  3   │ │  4   │
└──────┘ └──────┘ └──────┘ └──────┘
```

```
┌─────────────────────────────────────────────────────────────────┐
│  Cart ID:                                                         │
│  0 - 255                                                          │
└─────────────────────────────────────────────────────────────────┘
```

The Cart ID is site specific: Phoenix MAC PC = 43
                                  Phoenix MAC 12 = 44
                                  Oklahoma MAC PC 1 = 48 (Lawton)
                                  Oklahoma MAC PC 2 = 49 (Anadarko)
                                  Rapid City: Eagle Butte = 59
                                                 Pine Ridge = 60
                                                 Fort Totten = 61

```
┌─────────────────────────────────────────────────────────────────┐
│  Site ID: 3                                                       │
│  1 - 255                                                          │
└─────────────────────────────────────────────────────────────────┘
```

```
┌─────────────────────────────────────────────────────────────────┐
│  Institution Name:      Strong Heart Study                        │
│  Up to 40 characters                                              │
└─────────────────────────────────────────────────────────────────┘
```

```
┌─────────────────────────────────────────────────────────────────┐
│  Number of Patient ID Digits:       11                            │
│  1 - 12                                                           │
└─────────────────────────────────────────────────────────────────┘
```

| Height / Weight: | | cm/kg | | | This may be omitted in the SHS. |
| in /lb | cm/kg | | | | |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

| Input Patient Age As : | DOB | | | = Date Of Birth. |
| DOB | Years | | | |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

| Ask Blood Pressure Questions: NO | | | | NO |
| Yes | No | | | |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

| Ask Options Question: | NO | | | NO |
| Yes | No | | | |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

| Suppress Normal Statement: | NO | | | NO |
| Yes | No | | | |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

| Suppress Border & Abnormal Statement: | | NO | | NO |
| Yes | No | | | |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

| ECGs to Store/Transmit: | All ECGs | | | ALL |
| All | Abnormal | | | |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

| Delete ECGs After Transmission: | SAVE |
| Save   Delete | |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

SAVE. It is very important to change this to SAVE. By default the machine deletes ECGs as soon as they are transmitted, without waiting for confirmation from Halifax.

| Store/Transmit Control: Store |
| Store   Transmit |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4· |

Store

| Power Up Speed: 25mm/s |
| 25mm/s   50mm/s |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

25mm/s

| Power Up Filter: 100 Hz |
| 40 Hz   100 Hz |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

100 Hz

| Screening Criteria: NO |
| Yes   No |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

NO

| Baseline Roll Filter: .16 Hz |
| .01Hz   .02Hz   .16Hz   .32Hz |

| F1 | F1↑ | F2 | F2↑ | F3 | F3↑ |
| 1 | 2 | 3 | 4 | 5 | 6 |

.16 Hz

| QC Baseline Drift: NO |
| Yes   No |

| F1 | F1↑ | F2 | F2↑ |
| 1 | 2 | 3 | 4 |

NO

```
QC Muscle Tremor:     NO
Yes               No
```

F1	F1↑	F2	F2↑
1	2	3	4

NO. Press ⬅ to

return to the *Main Menu*.

Step H: Defaults Setup

```
                        Cart Setup
Modem          Passwds      Misc    Defaults      More
```

F4	F4↑
7	8

⬇

```
Are you Sure???
yes             No
```

Never say yes to return
original factory setup
defaults, because that will
set the machine to delete
ECGs after transmission.
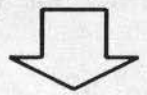
F1	F1↑	F2	F2↑
1	2	3	4

⬇   ⬇
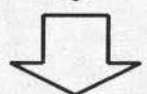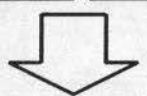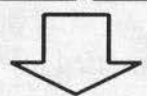
⬇   Return LCD to the following display.

Return the MAC PC to its original factory setup defaults.
Any setup changes that you made will be lost..

Setup I: Timeout Setup

```
               Cart Setup
Modem          Passwds      Misc    Defaults      More
```

F5	F5↑
9	0

⬇
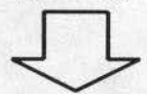
```
┌────────────────────────────────────────────────────────────┐
│                    Cart Setup                                │
│   Timeout          RS232          Monitor More               │
└────────────────────────────────────────────────────────────┘

┌──────┐ ┌──────┐
│  F1  │ │ F1↑  │
│  1   │ │  2   │
└──────┘ └──────┘

         ⬇

┌────────────────────────────────────────────────────────────┐
│   Automatic Timeout (minutes):           1 0                 │
│   1        5       10      30      none(ac)                  │
└────────────────────────────────────────────────────────────┘     This saves the battery.

┌────┐┌──────┐┌────┐┌──────┐┌────┐┌──────┐┌────┐┌──────┐┌────┐┌──────┐
│ F1 ││ F1↑  ││ F2 ││ F2↑  ││ F3 ││ F3↑  ││ F4 ││ F4↑  ││ F5 ││ F5↑  │
│ 1  ││  2   ││ 3  ││  4   ││ 5  ││  6   ││ 7  ││  8   ││ 9  ││  0   │
└────┘└──────┘└────┘└──────┘└────┘└──────┘└────┘└──────┘└────┘└──────┘
```

Select F1 to set a 1-minute timeout, F2 to set a 5-minute timeout, F3 to set a 10-minute timeout, F4 to set a 30-minute timeout, or F5 to set an indefinite timeout length.

"Timeout" is the amount of time it takes for the LCD to go blank when the MAC PC is not being used.
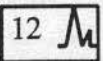
If the "none(ac)" is selected, the timeout length will be indefinite only if a power module is attached to the MAC PC and the battery status (section 12) message indicates "OK" or "FULL." Otherwise, if a power module is NOT attached, then the timeout length will be set to 10 minutes.

```
┌────────────────────────────────────────────────────────────┐
│                    Cart Setup                                │      Press   ⊘      to
│   Timeout          RS232          Monitor      More          │      return to the Main Menu.
└────────────────────────────────────────────────────────────┘
```

APPENDIX 12(b)   Taking a Resting ECG
Entering Patient Information

Note: It is NOT necessary to enter any patient information in order to take a resting ECG.
You can record an ECG at any time -- if the *Main Menu* is displayed by just pressing [12 ∿]
If you do not enter the patient's name and identification number, the patient will be
identified by the date and time when the ECG was taken.

Note: When a patient's age is entered and the patient is 15 years old or less, then a pediatric
12SL analysis is performed on the ECG data. However, if NO age is entered, then the
MAC PC will always performed an adult analysis.

If the Main Menu is not already displayed, then press   ⊘   to return to it:

```
↑Task  V1 + II + V5
PatInfo    Rhythm      25mm/s    10mm/mv    100Hz
```
Hit either F1 or F1↑.

```
F1      F1↑
1       2
```

Next, press either F1 or F1↑ to select PatInfo (F1). One of the following two prompts will appear:

```
Patient Last name:
A to Z, Space.
```
Enter names.

OR

```
New Patient:
Yes              No
```
This won't show up if the
machine was just turned on.
Hit either F1 button if it is a
new person. Hit either F2
button if you want to
correct an entry and/or take
another ECG on the same
person.

```
Patient First Name:
A to Z, 0 to 9, Space.
```

```
Patient ID:
Digits 0 to 9.
```
This is actually an 11 digit
ID. Enter five (5) 0
followed by six (6) digits
Strong Heart Study ID.

```
Referred By :
(Physician Name)
```
Skip over [⏎]

Location Number: 0 to 99

Skip over ⟵

Room Number: Any 5 characters

This is the interviewer #.
Enter your SHS code here.

Date of Birth (DD - MM -- YY) DD = Day, MM=Month, YY=Year

To type dash press ⬦

and [- F] at the same

time. MM=letters of month
(JAN, FEB, ...,etc.) YY=
Year (89, 90, etc.)

Height: (in cm)

Skip over ⟵

Weight: (in kg)

Skip over ⟵

Sex: Male   Female

Indicate Sex. Hit either F1
F2.

Race: Cauc   Black   Oriental   Hisp   More

Choose "More". Hit either
F5.

F5	F5↑
9	0

⬇

Race: Unknown   Indian

Choose "Indian". Hit either
F3.

F3	F3↑
5	6

⬇

Medication: none   Unknown   Add   Scroll

If on CARDIAC medication
enter either F4. Find medi-
cation and enter. (This is
not part of Strong Heart
Study essential information)

F4	F4↑
7	8

The MAC PC is now ready to take a 12-lead ECG. Press [12∿] to start.

Fault Detection Procedures

Should problems with noise or drift be encountered, electrodes are replaced. The following is a guide for determining which electrodes may be faulty. The underlined electrodes are the predominant determinants of the appropriate lead and therefore are most likely to be the faulty electrodes for a given lead. After adjustment or replacement of suspect electrodes, the electrocardiograph should be able to record 10 seconds of good data.

Lead Affected	Possible Faulty Electrode
I	RL, RA, LA
II	RL, RA, LL
III	RL, LA, LL
aV_R	RL, RA, LL, LA
aV_L	RL, LL, RA, LA
aV_F	RL, LL, RA, LA
V_1	RL, LL, RA, LA, V_1
V_2	RL, LL, RA, LA, V_2
V_3	RL, LL, RA, LA, V_3
V_4	RL, LL, RA, LA, V_4
V_5	RL, LL, RA, LA, V_5
V_6	RL, LL, RA, LA, V_6

Self-Evaluation of Technical Performance

This section allows technicians to monitor their own ECG technique. It is intended to help technicians who are having difficulty meeting the quality standards set by the ECG Reading Center. These data are not intended to be collected by the study.

The technician examines the ECG tracing to estimate the noise level and baseline drift. Based on the requirements of the Minnesota Code, acceptable and unacceptable levels of noise and baseline drift have been established. These levels are scored using the following table:

Noise Grade	Overall (mm)	Beat-to-beat Drift (mm)	Quality Drift (mm)
1	< .25	< 1	< 1
2	< .50	< 2	< 1.5
3	< 1	< 3	< 2
4	< 2	< 4	< 3
5	> 2	> 4	> 3

The grade levels given in this table are related to the ability of the analysis program to achieve the required accuracy. Quality Grade 5 is unacceptable. ECGs of Quality Grade 5 must be deleted from the machine's memory and retaken immediately.

1. First, the tracing is examined for obvious errors such as right arm/left arm and other common lead misplacement (see Figure 4, negative p-waves in I indicate lead switch). These ECGs must be deleted from the machine's memory and retaken immediately.

2. The Quality Grade for noise is obtained by measuring the noises level ss vertical peak-to-peak values in terms of number of small paper divisions (smallest grid squares). Note that recording sensitivity is 1 mV per centimeter, (one small paper division = 1 mm = 0.1 mV). A noise level of more than 2 small paper divisions (> 0.2 mV peak to peak) is unacceptable (Figure 5).

3. The Quality Grade for overall drift is obtained by searching each of the 12-leads for the maximum and minimum baseline levels within that lead (as determined by the PR and/or TP segments) over the 10 second recording and measuring the vertical distance between them. A distance of more than 4 small paper divisions is unacceptable (Figure 6).

4. The Quality Grade for beat-to-beat drift is determined by searching for the pair of successive QRS complexes having the largest amplitude difference (vertical distance) between successive PR segments. A difference of more than 3 small paper divisions (> 0.3 mV) indicates an unacceptable record (Figure 7).

Improvement in technical quality will indeed result if the prescribed procedure for electrode position marking, electrode and skin preparation, electrode replacement and equipment use are carefully followed. Baseline drift problems, which are essentially caused by poor electrode-skin contact are particularly easy to remedy, as is 60-cycle interference.

Sixty-cycle interference is characterized by perfectly regular fine oscillations occurring at the rate of sixty per second (Figure 8).

Electrical equipment of any kind may be the source of AC interference on an ECG in all leads or only certain ones. Check quality of skin preparation and electrode contact. Check leadwires and resecure attachment of the alligator clip to the electrode. Make sure participant does not touch any metal part of the bed or other equipment. Proximity to a wall with hidden wiring or a partially broken cable may also cause this problem.

Muscle Tremor causes irregular oscillations of low amplitude and varying rapidity superimposed upon the ECG waveform (Figure 9). Muscle tremor is the involuntary muscle activity of a participant whose state is tense, apprehensive, or uncomfortable. This is why a clear explanation of the electrocardiogram test and reassurance are necessary for the participant. The participant is asked if the temperature.of the room is too low for her/him and is covered with a blanket if so.

Original Hard Copy Record

The original 12-lead ECG record is filed at the field center. If the clinic needs a second "original" ECG, it can be printed from the machine's memory anytime before deletion of the ECG. The first hard copy ECGs are read locally by clinic physicians for notification and referral if needed. The records are then placed in participants' local data files. Double-check that this participant is correctly identified.
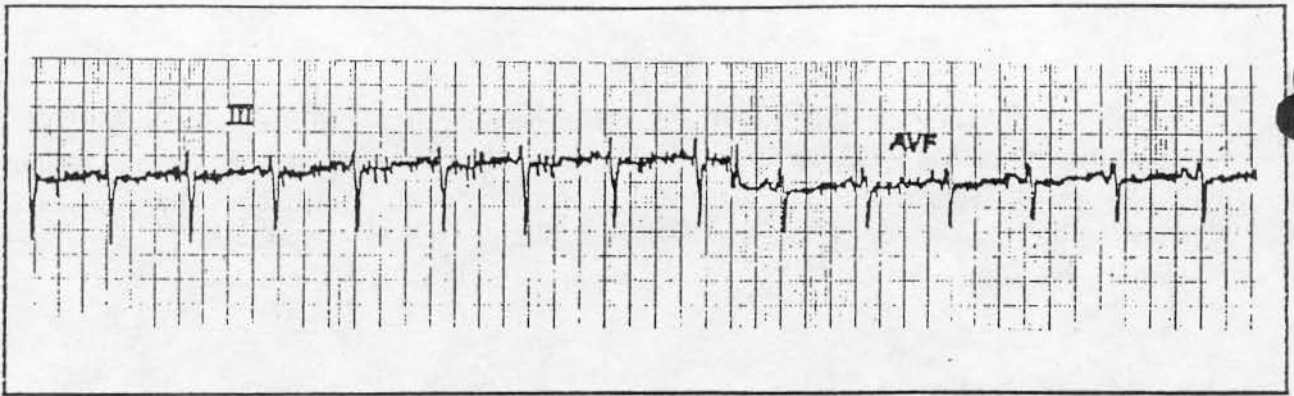
Figure 5.   Unacceptable Noise Level
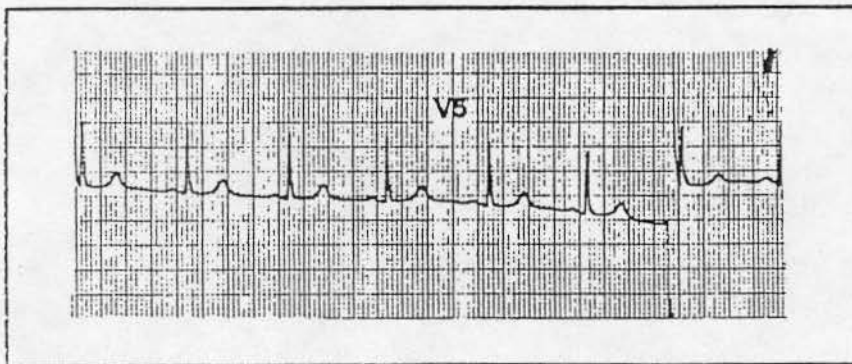
Figure 6.   Unacceptable Overall Baseline Drift
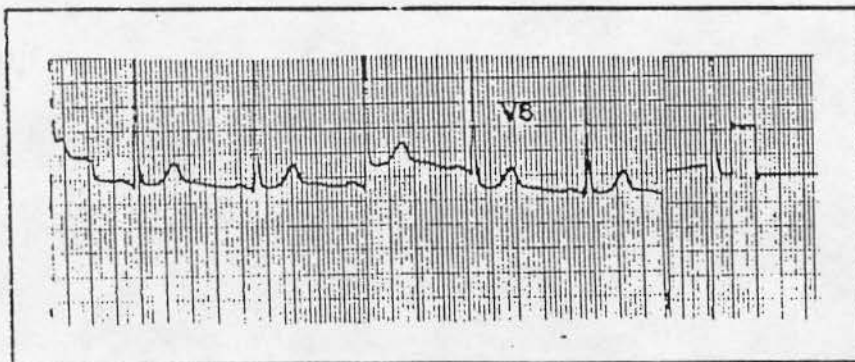
Figure 7.   Unacceptable Beat-to-Beat Baseline Drift
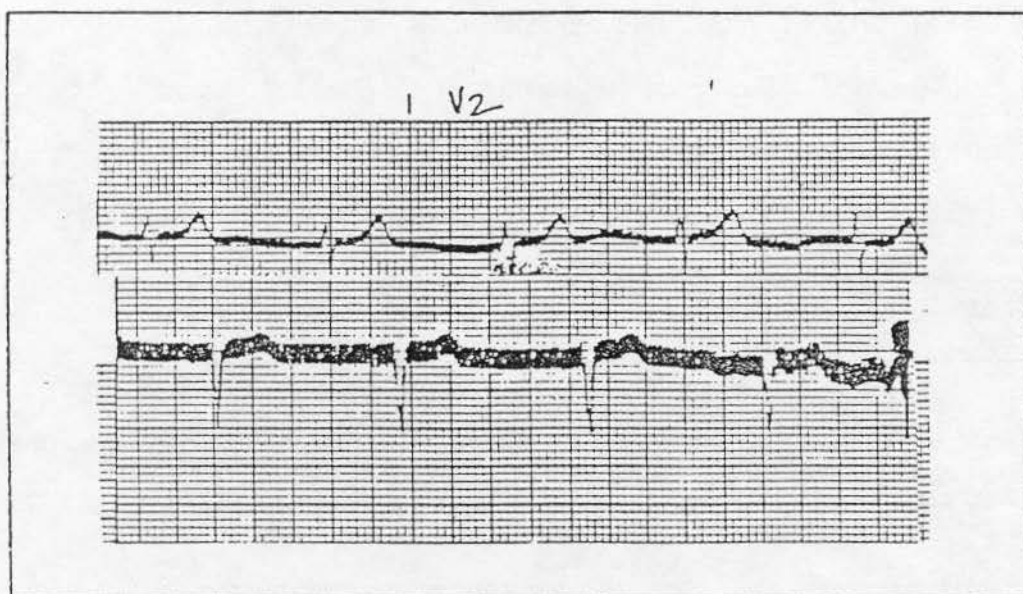
Figure 8.   Sixty-Cycle Interference
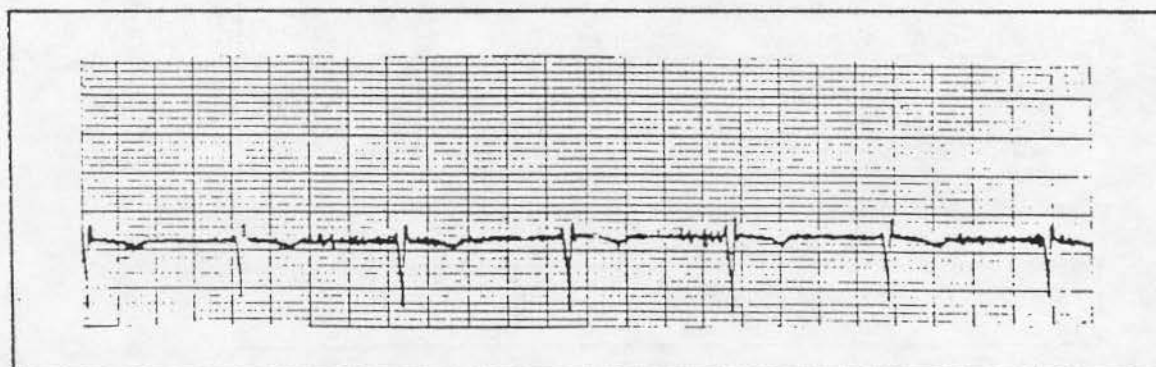
Figure 9.   Artifact Caused by Muscle Tremor

Appendix 12 (c) Transmitting ECGs by Telephone

Note: Only a MAC PC equipped with a modem can transmit ECG reports by telephone.

1. Prepare the MAC PC as described in section 1.

2. Connect a telephone cord from a telephone wall jack to the backpanel jack on the MAC PC.

3. If the Main Menu is not already displayed, press ⊘ :

```
↑Task          V1 + II + V5
PatInfo        Rhythm          25mm/s          10mm/mv   100Hz
```

4. Press ⬘ and F1↑ to display the System Functions menu. Then press one of the two keys listed under each of the following displays:

```
                    System Functions
Storage    Setup    Diag    RevXmit    Monitor
```

F1	F1↑
1	2

⬇

```
                    Storage Functions
Plot    Directory    Summary    Delete    More
```

F5	F5↑
9	0

⬇

```
                    Storage Functions
Transmit        Edit        Format              More
```

F1	F1↑
1	2

⬇

```
              Transmission Type
Phone         Local          RS232
```

F1	F1↑
1	2
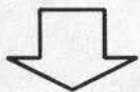
```
                    ** No Data in Storage **
```

OR

```
Phone Number:
0 - 9 # * = ,
```

5. If the second display appears, type in the phone number of the location where you will be

   transmitting and press ⬅ .

   The # and * are touch-tone symbols.

   The , sign provides a 2-second pause and may be used repeatedly for longer parses. (For example, in the phone number 1,,,8081112345 there will be a 6-second pause between the numbers "1" and "8" when dialing.)

   The = sign is used to wait for a dial tone. (For example, in order to dial an outside number, your phone system may require you to dial "9" first. A sample number would look like this: 9=1234567.)

6. Next, patient data on each stored ECG will be displayed similar to the following:

   Pressing No (F2)   Pressing Yes... (F4) selects this ECG
   Bypasses this ECG.   and all subsequent ECGs.

```
 123456789 │   ALLEN,        BRADLEY
     Yes        No        No...        Yes...         Expand
```

   Pressing Yes (F1)   Pressing No... (F3)   Pressing Expand (F3) provides additional
   selects this ECG.   bypasses this ECG and   patient information such as date and time
                            all subsequent ECGs.   of the ECG. Use this function to verify
                                                       which single ECG to save and transmit on
                                                       each participant.

7. To display additional patient information, press Expand (F5) and a message similar to the one below will be displayed:

```
a                          b                        c
┌─────────────────────────────────────────────────────────┐
│ 123456789        ALLEN,      BRADLEY                       │
│ E      U      01-JAN-86    12:13 C001  L001  S001   Cont  │
└─────────────────────────────────────────────────────────┘
  i      h              g        f     e     d
```

a.   Patient identification number.

b.   Last name, first name of patient or the date and time when ECG was recorded.

c.   Select to return to former display.

d.   MUSE site number where ECG was recorded.

e.   Location number where ECG was recorded.

f.   Cart number of the unit where ECG was recorded.

g.   Date and time of ECG acquisition. This is a unique identifier if more than one ECG was saved on a particular subject.

h.   A U means that the ECG is unconfirmed. An C means that the ECG is confirmed. Use the Edit function described in section 6 to change an unconfirmed ECG to a confirmed ECG.

i.   Type of Data. E stands for ECG.

8.   Depending on which ECGs you want to transmit or bypass, press the appropriate function (F) key.

9.   After selecting the ECGs you want to transmit, displays similar to the following will appear:

```
┌─────────────────────────────────────────────────────────┐
│              ** Batch Transmission **                     │
│ Waiting for Dial Tone                                     │
└─────────────────────────────────────────────────────────┘
```

THEN

```
┌─────────────────────────────────────────────────────────┐
│              ** Batch Transmission **                     │
│ Dialing 1112345                                           │
└─────────────────────────────────────────────────────────┘
```

THEN

```
┌─────────────────────────────────────────────────────────┐
│              ** Batch Transmission **                     │
│ Waiting for an Answer Tone                                │
└─────────────────────────────────────────────────────────┘
```

THEN

```
┌─────────────────────────────────────────────────────────┐
│              ** Batch Transmission **                     │
│ 123456789          JONES,       JACK                      │
└─────────────────────────────────────────────────────────┘
```

10.   After the last ECG has been transmitted, a message indicating the number of ECGs that were transmitted vs the number you selected to transmit will be displayed similar to the following:

```
┌─────────────────────────────────────────────────────────┐
│         5  of  5  Transmitted                             │
│ Type  Any  Key  to  Continue                              │
└─────────────────────────────────────────────────────────┘
```

11. Pressing any key displays the following:

	Transmission Type	
Phone	Local	RS232

Press to return to the Main Menu.

12. If, despite previously mentioned safeguards, you have still erroneously transmitted a tracing with improper ID #, time, or a non-SHS ECG, please FAX this data to Dr. Oopik immediately at (303) 674 - 4196.

13. All transmitted ECGs should be logged at the study field clinic.

A copy of this log page should be faxed to Dr. Oopik weekly on Monday to verify ECG authenticity before ECGs are sent to Minneapolis.

Erroneous ECGs consumed much time during Phase I.

SHS ECG TRANSMIT LOG

Patient ID #	Date (from ECG)	Time (from ECG)	Patient Name

Appendix 12 (d)   Receiving ECGs by Telephone
Receiving By Telephone

Note: Only a MAC PC equipped with a modem can receive ECG reports by telephone.

Note: If 75% or more of the MAC PC's memory is used, then the message "Plotter Output Only" will appear. This means that incoming data will be printed but NOT stored. In this case, if you want to store incoming data, then delete some ECGs from the MAC PC *before* you begin receiving data (refer to section 9).

1.   Prepare the MAC PC as described in section 1.

2.   Connect a telephone cord from a telephone wall jack to the backpanel jack on the MAC PC.

3.   If the Main Menu is not already displayed, press   ⊘   :

```
↑Task          V1 + II + V5
PatInfo     Rhythm     25mm/s     10mm/mv     100Hz
```

4.   Press   ⊗   and F1↑ to display the System Functions Menu:

```
                    System Functions
Storage      Setup      Diag      RevXmit      Monitor
```

```
 F4      F4↑
  7        8
```

5.   Select RevXmit (Reverse Transmission) to display:

```
                    Transmission Type
Phone          Local          RS232
```

```
 F1      F1↑
  1        2
```

6.   Select Phone (F1) and one of the following two message will appear:

```
No Data Storage - Plotter Output Only
Type Any Key to Continue
```

OR

```
Select Option:
        Store          Plot
```

```
 F1      F1↑      F2      F2↑
  1        2        3        4
```

7.   If the second display appears, select Store (F1) to store and print out the ECG(s) you receive, or select Plot (F2) to just print out the ECG(s) without storing them. Then a display similar to the following will appear:

```
              ** Reverse Transmission **                      d
Check the Phone Line
```

8.   If the following message appears, then the telephone line is not attached:

```
              ** Reverse Transmission **
Phone Line Not Attached
```

9.   Otherwise, the following series of message will be displayed for each ECG that is received:

```
              ** Reverse Transmission **
Ready to Receive
```

THEN

```
              ** Reverse Transmission **
Answer the Phone
```

THEN

```
              ** Reverse Transmission **
Receiving Data
```

THEN

```
              ** Reverse Transmission **
End of Data Packet
```

THEN

```
              ** Reverse Transmission **
Page xx of xx
```

10. After all ECGs have been received, following will appear:

```
              ** Reverse Transmission **
End of Transmission
```

THEN

```
              ** Reverse Transmission **
Ready to Receive
```

11. If no other ECGs will be received, then press [⊘] to return to the Main Menu. NOTE:
Use the Directory function (section 8) to check that all ECGs have been received.

APPENDIX 12 (e)   Deleting an ECG

Since most ECG storage is only temporary, there will probably be times when you want to delete recordings from the MAC PC's memory. Also, there may be times when the memory is almost full, and the MAC PC itself suggests that you delete ECGs. (Refer to the Section on "Forced Deletion".) ECGs taken in the Strong Heart Study should be kept in memory until confirmed copy is returned. The machine will not automatically delete ECGs except that procedures are carried out as described in "Forced Deletion".

Routine Deletion

ECGs are usually deleted after you print a paper copy of the ECG, when more than one ECG per patient has been stored or when the ECG is transmitted to another location. To delete one or more ECGs, follow these steps:

1.   Prepare the MAC PC as previously described.

2.   If the Main Menu is not already displayed, press ⬡ :

↑Task	V1 + II + V5			
PatInfo	Rhythm	25mm/s	10mm/mv	100Hz

3.   Press ⬖ and F1↑ at the same time to display the System Functions menu. Then press

one of the two keys listed under each of the following displays:

		System Functions		
Storage	Setup	Diag	RevXmit	Monitor

F1	F1↑
1	2

⬇

		Storage Functions		
Plot	Directory	Summary	Delete	More

F4	F4↑
7	8

4.   After selecting Delete (F4) a message similar to the following one will be displayed:

Pressing Save (F2) saves this ECG.

Pressing Expand (F5) provides additional patient information such as date and time of the ECG.

5%	123456789	ALLEN,	BRADLEY	
Delete	Save	Save...	Quit	Expand

Pressing Delete (F1) Deletes this ECG.

Pressing Save... (F3) saves this ECG and all Subsequent ECGs.

Pressing Quit (F4) leaves the Delete function.

5. To display additional patient information, press Expand (F5) and a message similar to the one below will be displayed:

```
a           b                          c                        d
┌─────────────────────────────────────────────────────────────────┐
│ 5%    123456789         ALLEN,        BRADLEY                      │
│ E     U     23-MAR-88   09:53  C001  L001  S001  Cont             │
└─────────────────────────────────────────────────────────────────┘
   j     i                  h        g      f      e
```
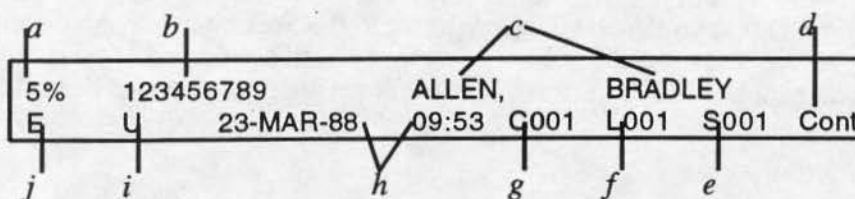
a. Percentage of memory used by this ECG.

b. Patient identification number.

c. Last name, first name of patient or the date and time when ECG was recorded.

d. Select to return to former display.

e. MUSE site number where ECG was recorded.

f. Location number where ECG was recorded.

g. Cart number of the unit where ECG was recorded.

h. Date and time of ECG acquisition. This is a unique identifier if more than one ECG was saved on a particular subject.

i. A U means that the ECG is unconfirmed An C means that the ECG is confirmed. Use the Edit function described in section 6 to change an unconfirmed ECG to a confirmed ECG.

j. Type of Data. E stands for ECG.

6. Depending on what you want to delete, save, or bypass, press the appropriate function (F) key.

7. After you have decided which ECGs you want to delete, you have another chance to change your mind. For instance, if you have decided to delete two ECGs, this message would be displayed:

```
┌─────────────────────────────────────────────────────────────────┐
│ Delete          2 ECG(s) ? :                                      │
│         Yes             No                                        │
└─────────────────────────────────────────────────────────────────┘

┌──────┐ ┌──────┐ ┌──────┐ ┌──────┐
│ F1   │ │ F1↑  │ │ F2   │ │ F2↑  │
│ 1    │ │ 2    │ │ 3    │ │ 4    │
└──────┘ └──────┘ └──────┘ └──────┘
```

Cancels the Delete.

Deletes the selected ECGs.
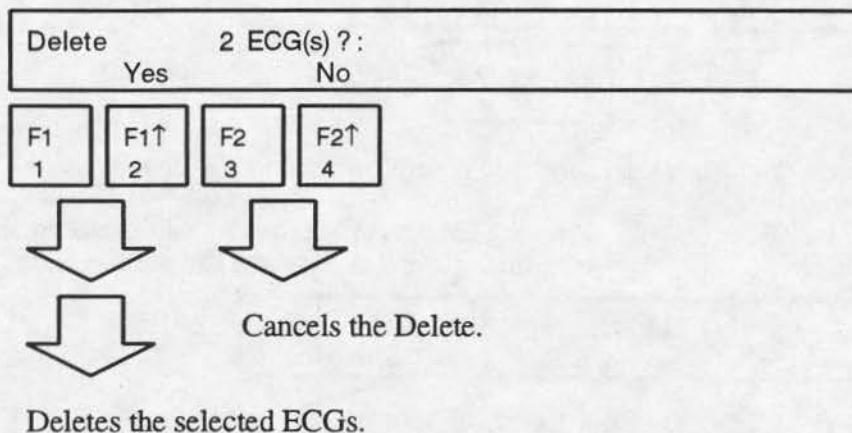
If the ECG you are recording requires more memory than the MAC PC is able to spare, a prompt will appear after the Processing ECG for Storage display:

```
ECG storage: Insufficient Space Available
Type Any Key to Continue
```

1.   Pressing any key to continue causes this message to be displayed:

```
Select Option:
         Delete          Quit          Xmit
```

```
| F1 |  | F1↑ | F2 |  | F2↑ | F3 |  | F3↑ |
|  1 |  |  2  |  3 |  |  4  |  5 |  |  6  |
```

- Select Delete (F1) to display one of the ECGs stored in the MAC PC's memory. A small explanation of how each function key affects ECGs stored in the MAC PC follows.

- Select Quit (F2) to return to the "ECG Storage: Insufficient Space Available" display.

- Select Xmit (F3) if you want the MAC PC to transmit the ECG you just acquired instead of storing it. (Xmit will only appear if your MAC PC is equipped with a modem.)

2.   If you select Delete, a display similar to the following will appear:

Pressing Expand (F5) provides additional patient information such as date and time of the ECG.

```
( 5%)    24%    123456789    ALLEN,        BRADLEY
Delete           Save         Quit          Expand
```

Pressing Delete (F1)   Pressing Save (F2)   Pressing Quit (F4) return to the
deletes this ECG.   saves this ECG.   "Insufficient Storage" display.

3.   To display additional patient information, press Expand (F5) and a message similar to the one below will be displayed:

a   *b*   *c*   *d*   *e*
```
( 5%)   24%       123456789        ALLEN,     BRADLEY               
E       U         23-MAR-88    09:53  C001    L001    S001    Cont
```
k   *j*   *i*   *h*   *g*   *f*

a. Percentage of storage that must be deleted to provide room for the ECG just acquired. This number decreases as ECGs are selected for deletion.	*g.* Location number where ECG was recorded.
	h. Cart number of the unit where ECG was recorded

b. Percentage of memory used by this ECG.

c. Patient identification number.

d. Last name, first name of patient or the date and time when ECG was recorded.

e. Select to return to former display.

f. MUSE site number where ECG was recorded.

i. Date and time of ECG acquisition. This is a unique identifier if more than one ECG. was saved on a particular subject.

j. A U means that the ECG is unconfirmed An C means that the ECG is confirmed. Use the Edit function described in section 6 to change an unconfirmed ECG to a confirmed ECG.

k. Type of Data. E stands for ECG.

4. After you have either Saved or Deleted all stored ECGs, one of the following two displays will appear:

```
Not enough ECG(s) selected for deletion
Type Any Key to Continue
```

OR

```
Delete  2  ECG(s) ? :
        Yes            No
```

F1	F1↑	F2	F2↑
1	2	3	4

If the first display appears, then you will return to the "ECG Storage: Insufficient Space Available" display. In this case, you will have to start the deletion process all over.

If the second display appears, or one like it, then select Yes (F1) to delete the selected ECGs or No (F2) to return to the "ECG Storage: Insufficient Space Available" display.

5. If you selected Yes (F1), the ECG you just recorded will be stored. The this message will be displayed:

```
            ** ECG Storage Complete **
Type Any Key to Continue
```

Pressing any key to return you to the *Main Menu*.

APPENDIX 13
THE STRONG HEART STUDY II

STANDARD ECG INSTRUCTIONS

1.   BASELINE ECGs

1.1   Introduction

During the baseline examination, a standard supine 12-lead resting ECG is recorded at least one half hour after ingestion of glucose.

1.2   Procedure for Recording Baseline ECG

The standard electrocardiograph for the Strong Heart Study is the MAC PC Personal Cardiography by Marquette Electronics, Inc. The standard configuration for the MAC PC is shown in Appendix A. A 12-lead resting ECG tracing is obtained consisting of 2.5 seconds of each of the leads simultaneously (I, II, III, aV_R, aV_L, aV_F, V_1-V_6) with a 10 second lead Rhythm Strip.

Procedures for charging the battery of the MAC PC: The MAC PC runs only from its battery. The battery can be charged by plugging the unit into a wall outlet. The MAC PC will record and print about 50 ECGs on one charge. The amount of charge left is displayed for one-half second when the machine is turned on. It takes about 10 hours to charge the battery.

Plug in the unit each evening after transmitting data to Fitzsimmons. Unplug the unit in the morning. It is not good for the machine to spend several days in either the fully charged or completely drained state. For weekends and holidays the machine may be left plugged in, or, if the brief charge display shows at least 25 ECGs remaining, it may be left unplugged.

1.3   Electrode Position Measuring and Marking

Because it is essential for the study to be able to compare baseline ECG data with subsequent records, a uniform procedure for electrode placement and skin preparation is required. The method and procedure for standardizing electrode locations are outlined below.

The participant, stripped to the waist, is instructed to lie on the recording bed with arms relaxed at the sides. The individual is asked to avoid movements which may cause errors in marking the electrode locations, but encouraged to converse with the technician. Prior experience with electrocardiograms is discussed, as is the purpose of the ECG recording. The participant should be told this is a research ECG to be used for statistical analysis later in the study. However, it can also be used by the clinic physician for general diagnostic purposes, and a copy can be sent to the individual's private physician.

For best electrode/skin interface, place the electrodes on the skin at least 2-3 minutes before taking the ECG. Patient information can be entered on the MAC PC during this time. This waiting time is not so critical with the suction electrodes, if it is anticipated that data entry will take > 3 minutes, you may want to enter data first when using these electrodes. It is recommended that the stick-on electrodes be applied 2-3 minutes prior to acquiring the ECG.

A good felt tip pen is used to mark the six chest electrode positions. Wipe the general area of the following 10 electrode sites with a sterile alcohol prep to remove skin oil and perspiration. It is extremely important that care be taken to locate these positions accurately. Therefore, the procedure given below must be meticulously followed. Electrode positions in women with large, pendulous breasts must be determined in relation to the anatomic points described below - as for all participants. The electrodes must then be placed on top of the breast (in the correct position).

1.3.1 Chest Leads (Figure 1)

1. Electrode V_2

Locate the sternal angle and second left rib between the index and middle fingers of your right hand. Count down to the fourth rib and identify the fourth intercostal space below it. Locate V_2 in the fourth intercostal space immediately to the left of the sternal border.

2. Electrode V_1

Locate electrode V_1 in the fourth intercostal space at the right sternal border. This should be at the same level as V_2 and immediately to the right of the sternum.

3.   Anterior 5th Interspace Marker (E Point)

Identify the fifth rib and fifth intercostal space below V_2 by counting down ribs as described for V_2. Follow this space horizontally to the midsternal line and mark this point. This is the "E" point.

4.   Electrode V_6

Locate the V_6 electrode at the same level as the E point in the midaxillary line (straight down from the center of the armpit). If breast tissue is over the V_6 area, mark the V_6 location on the breast.

Do not attempt to move the breast in order to mark V_6 on the chest wall, **unless** doing so is absolutely necessary to achieve better anatomic position.

5.   Electrode V_4

Electrode V_4 is located using the E-V_6 Halfpoint Method. Using the medical tape measure employed in anthropometry, measure the distance between the E point and the V marking. The tape should be resting lightly on the skin, not pressing into the flesh. The E and V_6 marks should be clearly seen. Place electrode V4 midway between E and V_6.

6.   Electrode V_3

Using the medical tape measure employed in anthropometry, mark the location of electrode V_3 midway between the locations of V_2 and V_4.

7.   Electrode V_5

Using the medical tape measure employed in anthropometry, mark the location of electrode V_5 midway between the locations of V_4 and V_6.
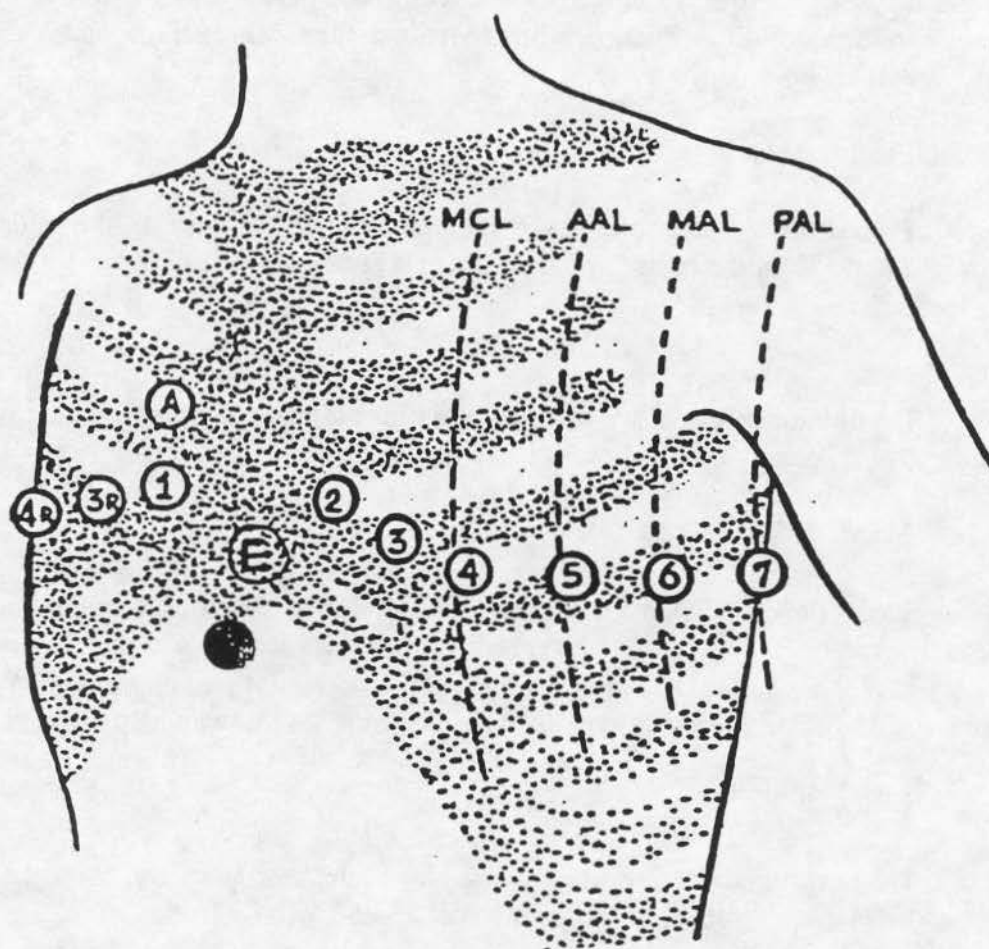
Figure 7. Precordial points from which chest leads are derived
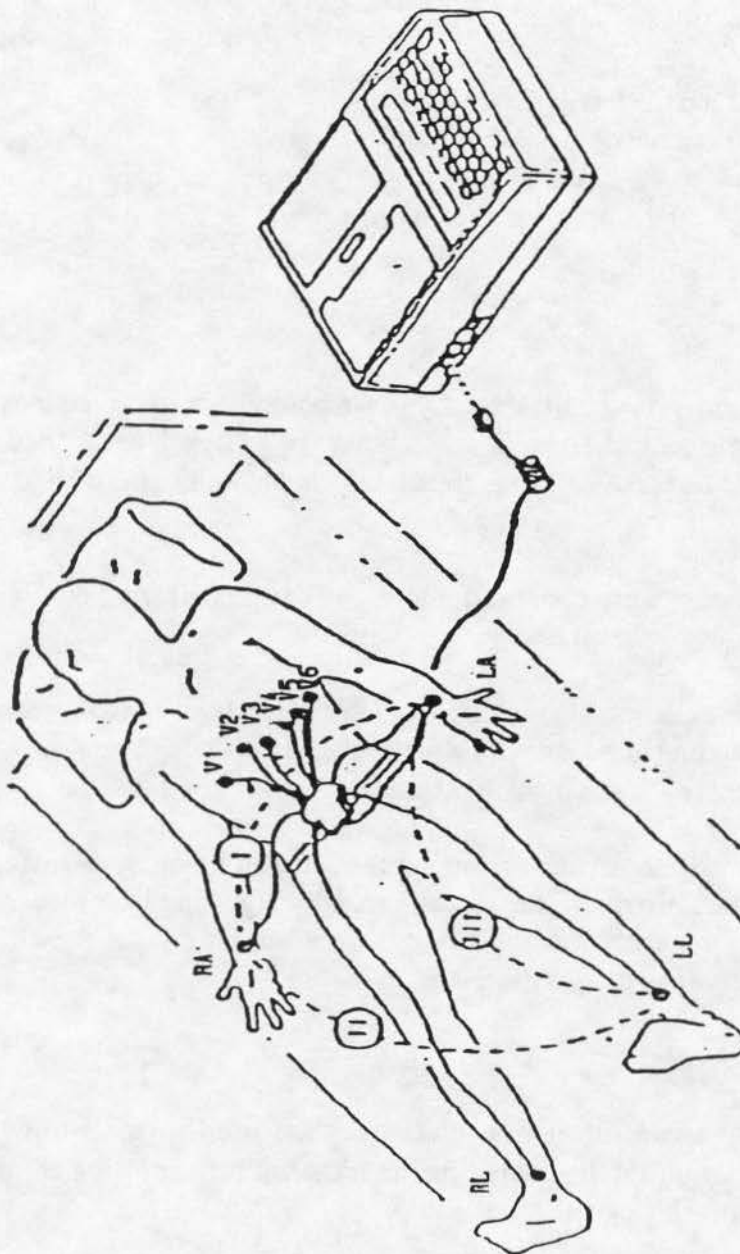
Figure 8. Electrode and leadwire placement

1.3.2   Limb Leads (Figure 2)

Locate electrode LL on the left ankle (inside).
Locate electrode RL on the right ankle (inside).
Locate electrode LA on the left wrist (inside).
Locate electrode RA on the right wrist (inside).

1.4   Skin Preparation

Skin preparation is undertaken only in the presence of observed technical problems due to poor electrode contact. As a first step it may be sufficient to rub the skin lightly with a tongue depressor or piece of gauze to produce reddening. If this does not resolve the problem, then:

1.   With the participant's consent, remove any excess hair from each electrode site on the chest using a shaver.

2.   At each electrode location in turn the outer horny layer of the epidermis is removed by gentle dermal abrasion with a piece of gauze. Only three passes (in the form of an asterisk) at each site using light pressure are required.

If the skin preparation has removed the felt pen marking at any of the electrode sites, these are accurately re-established by carefully repeating the procedure described in Electrode Position Measuring and Marking. It is important that the electrode sites be marked using the exact technique described.

1.5   Application of Electrodes

Either disposable or suction electrodes are used in the Strong Heart Study. Adaptors are used with the leadwires to connect the "banana" plug from the MAC PC leadwire to the disposable electrode via a clip.

When placing each electrode, massage it in a small circular motion to maximize the pre-gel contact with the skin but avoid overlap of gel from one electrode to the next.

Center the four limb electrodes on the inside of the wrist or ankle with the tab for the clip pointing toward the head. Center the six chest electrodes on the chest markings with the tabs pointing down. Do not let the electrodes overlap or touch each other if possible.

Clip the appropriate leadwire to each electrode (Figure 1). Do not pull or jerk tangled wires. To untangle wires, disconnect lead wires from electrodes.

1.6   Recording the 12-lead ECG

Change the roll of paper as needed. Each roll is 75 feet long; each patient takes approximately one foot of paper.

Each ECG is automatically stored in memory until it is deleted. After placing the electrodes on the skin, enter the participant information into the MAC PC (Figure 3) according to Appendix B. Disposable electrodes particularly must be on the skin for at least 2-3 minutes before taking the ECG. Make a final check of the electrodes and lead wires. Ask the participant to relax and keep still, then press the RECORD key.

The machine will display "Acquiring Data" and the left side of the display will show a count. If there are technical problems the display will show which lead is involved and will keep counting until it gets 10 seconds of good data. Check electrode contacts and leadwires, then check the display again. If the display counts past 75, push the STOP key and remove the electrodes. Prepare the electrode sites as discussed in Skin Preparation and follow the above protocol for exact relocation of electrodes. Press RECORD ECG. The machine will tell you to "enter a new patient or press RECORD." Press RECORD ECG a second time to start the ECG. The machine will automatically print the ECG after it has acquired 10 seconds of good data (Appendix C).

Tear the ECG off the machine and file it in your records.

Press RECORD ECG. The machine will tell you to "enter a new patient or press RECORD."
Press RECORD ECG a second time to start the ECG. The machine will automatically print the ECG
after it has acquired 10 seconds of good data (Appendix C).

Tear the ECG off the machine and file it in your records.
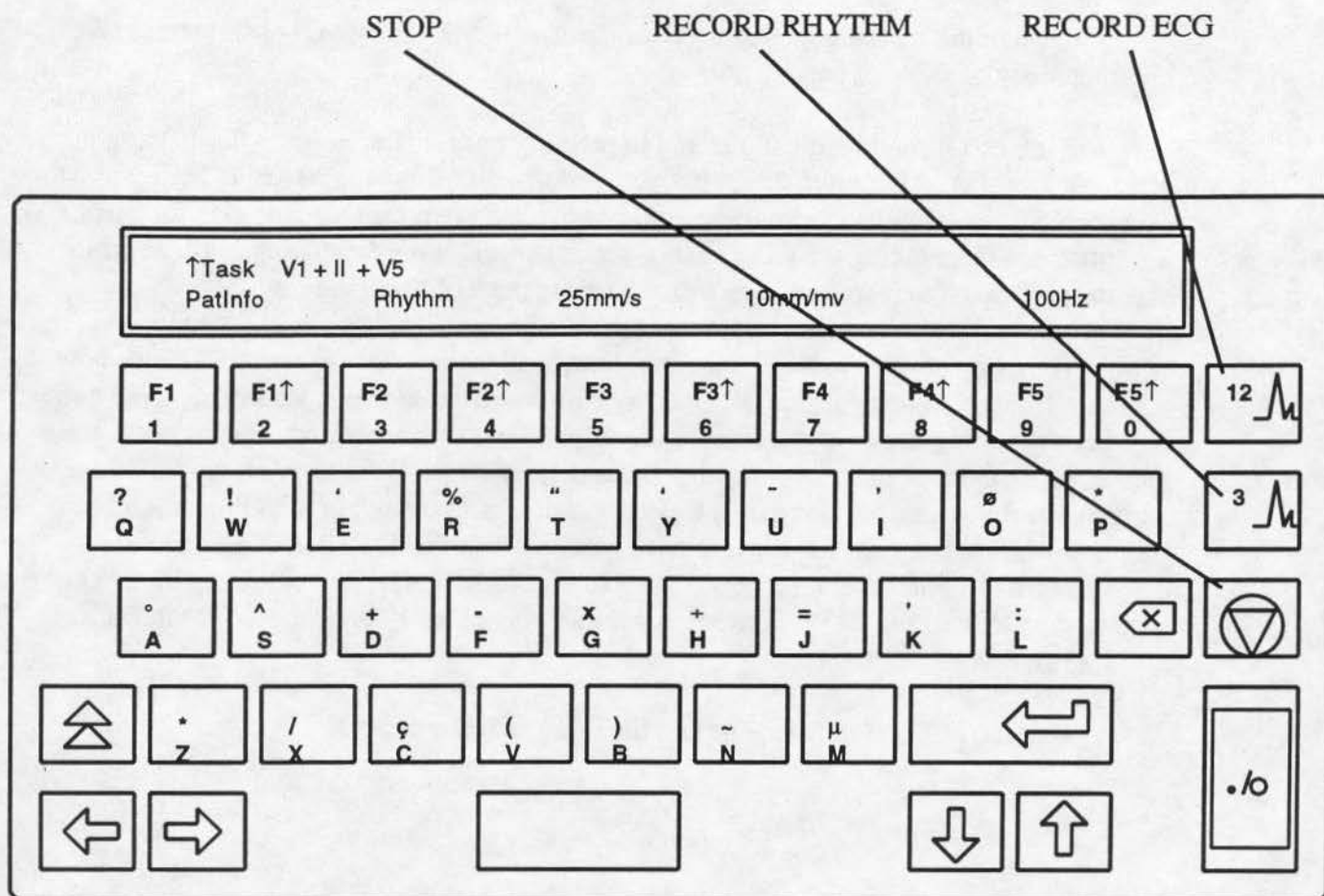
Figure 3. The MAC PC Keyboard and LCD Display by Marquette Electronics Inc.

THE STRONG HEART STUDY II

PHYSICIAN REFERRAL FORM FOR DIAGNOSIS OF CONGESTIVE HEART FAILURE

ID Number:

While Mr./Ms. _____ was participating in our examination, it appeared to us that he/she might have congestive heart failure. He/She thus has been referred to you for care. Listed below are the criteria that we are using for the identification of congestive heart failure in our study. We would very much appreciate it if you could complete the form below and send it to us, so that it may assist us in making the diagnosis. Record all that are present when you evaluate the patient.

I. Major Criteria	Present	Absent	Not evaluated
Paroxysmal nocturnal dyspnea or orthopnea	_____	_____	_____
Neck-vein distention	_____	_____	_____
Rales	_____	_____	_____
Cardiomegaly	_____	_____	_____
Acute pulmonary edema	_____	_____	_____
S_3 gallop	_____	_____	_____
Increased venous pressure >16 cm of water	_____	_____	_____
Circulation time $\geq$ 25 seconds	_____	_____	_____
Hepatojugular reflux	_____	_____	_____

II.   Minor Criteria   Present   Absent   Not evaluated

      Ankle edema   _____   _____   _____

      Night cough   _____   _____   _____

      Dyspnea on exertion   _____   _____   _____

      Hepatomegaly   _____   _____   _____

      Pleural effusion   _____   _____   _____

      Vital capacity decrease 1/3 from maximum   _____   _____   _____

      Tachycardia (rate of $\geq$ 120/min)   _____   _____   _____

III. Major/Minor Criteria   Present   Absent   Not evaluated

      Weight loss 4.5 kg in 5 days in response
      to treatment   _____   _____   _____

IV.   Tests that were performed on this patient.   (1=yes,   2=no)

      Echocardiogram   _____

      Chest x-ray   _____

      Measurements of vital capacity   _____

      Measurements of venous pressure   _____

In your opinion, does Mr./Ms. _____ have congestive heart failure?

If yes, what is the underlying cause? 1=yes   2=no
    (Please check the appropriate cause described below)

                  Valvular heart disease   _____

                  Atherosclerotic heart disease   _____

                  Cardiomyopathy   _____

                  Other   _____

                         please specify. _____

What is your specialty/subspecialty of medical practice?

We thank you very much for your assistance.

                  Signature_____Date _____

Referral Log

IDNO	Name	Date of Exam	Findings	Type of Referral	Refer to	Actions after Referral

THE STRONG HEART STUDY II

Sample Letter to Participant after Physical Examination

Dear _____:

Thank you very much for taking part in the Strong Heart Study on _____ (date). The final results of your blood tests and other measurements are now available and this letter summarizes the important findings from your visit:

Blood Pressure

Your blood pressure was _____ (less than 140/90 and not taking medication for BP). This is within the normal range. It should be checked at least once a year.

Your blood pressure was _____ (greater than 140/90). This is above the normal range. You should make an appointment for follow-up with your medical care provider, since high blood pressure may cause heart problems and stroke.

Your blood pressure was _____ (less than 140/90, taking BP medication). This is within the normal range. Continue taking your blood pressure medication as directed by your medical care provider.

Glucose Tolerance (Test for Diabetes)

Your fasting blood sugar was _____ (less than 140) and your 2 hour blood sugar was (less than 140). This is within the desirable range.

Your fasting blood sugar was _____ (less than 140) and your 2 hour blood sugar was (less than 200). These values are slightly high and raise the possibility that you may develop diabetes in the next several years. Weight reduction and exercise may help to prevent you from developing diabetes, so be sure to get advise from a health care provider on what might be helpful to you.

Your fasting blood sugar was _____ (greater than 140) and your 2 hour blood sugar was (greater than 200). These values are above the normal range and suggest that you may have diabetes. Please see your health care provider for advice on appropriate follow-up if you have not already done so.

Your fasting blood sugar was _____ (known diabetic less than 200). On the day of the exam, your fasting blood sugar was under good control. Be sure to follow the advice of your

medical care provider for control of your diabetes.

Your fasting blood sugar was _____ (known diabetic greater than 240). Your fasting blood sugar was higher than the usual target for diabetic patients. See your medical care provider for advice on how to attain better control.

Measurements of Blood Fats (Cholesterol and Triglycerides)

Your blood cholesterol was _____ (less than 200). This is within the desirable range, and we encourage you to maintain a healthy diet so that your cholesterol stays low.

Your blood cholesterol was _____ (200 to 240). This is slightly above the desirable range. We advise you to reduce the fat in your diet and have your cholesterol checked again in 6-months.

Your blood cholesterol was _____ (greater than 240). Your total cholesterol is high and this may cause heart problems. We advise you to reduce the fat in your diet and have your cholesterol checked again in 3 months.

Your blood triglycerides were _____ (less than 200). This is within the desirable range.

Your blood triglycerides were _____ (200-400). This is above the desirable range. We advise you to reduce your amount of calories and alcohol (if you are drinking) and have your triglycerides checked again in three months.

Your blood triglycerides were _____ (>400) This is a high value. You should see your health care provider for follow-up.

Electrocardiogram

We have sent a copy of your ECG (heart tracings) to your physician and he/she will notify you if there are any problems.

Echocardiograph

We have sent a report on your echocardiogram (movies of your heart's motion and flow of blood within it) to your physician and he/she will notify you if there are any problems.

Pulmonary Function

You blew air quickly into a spirometer to measure how well your lungs work. The results were an FEV1/FVC ratio of 68% and an FEV1 of 55% of the expected normal value.

[If the FEV1 is normal, above 80% predicted]

This means that your lungs are of normal size and your airways are wide open and move air normally.

[If both the ratio and FEV1 are abnormally low]

This means that you have airways obstruction. This could be due to cigarette smoking, asthma an other lung diseases. If you currently smoke cigarettes, stopping now will probably prolong your life. You may wish to discuss this with your physician during the next few months. [If the ratio is normal but the FEV1 is low]

This means that you can't take as deep a breath (or blow out as much air) as healthy people of your height. If you are overweight, this reduction in your vital capacity could be due to your weight, but your physician should examine your lungs and perhaps check your chest X-ray.

Body Fat

During the examination we measured the fat content in your body. The enclosed print out describes the results of this measurement, explains the normal values for your age and gender and suggests exercise programs and calorie recommendations.

Gallstones

The examination of your gallbladder did not show any gallstones.

The examination of your gallbladder showed that you have gallstones. If you are not having pain or other symptoms it is not necessary to seek any care at this time. However, we will include this information in your medical record and you should inform your doctor about the presence of the gallstones if your develop stomach cramps or other digestive problems. (Eliminate this section if the person has had gallbladder removed)

Skin Tests

Your skin test for tuberculosis was negative which suggests that you have not previously had or been exposed to tuberculosis.

You indicated that you had TB in the past and that you were treated for it. You don't need further evaluation for this unless you develop chronic cough, fever, weight loss or other symptoms.

Your skin test for tuberculosis was positive. This indicates that you were exposed to or have had tuberculosis in the past. If you have taken preventative treatment for 6-12 months, your

chances of getting TB are reduced. If you have not taken such treatment and are willing to take it, you should talk to your health care provider. This is especially important if you have had kidney or other organ transplants, diabetes, are currently undergoing dialysis.

Your skin test for valley fever was negative. This suggests that you have not previously had or been exposed to valley fever.

Your skin test for valley fever was positive. Although this means that in the past you were exposed to or had a case of valley fever, there is no need to receive any treatment unless you develop cough, fever, or weight loss, in which case you should see your healthcare provider. We will indicate this information in your medical chart so your health care provider will know about this test result.

The results of your tests will be sent to the IHS Hospital or Clinic as part of your hospital record. This will help the doctors take care of you if you get sick and go to the hospital or clinic. If your doctor does not work at the IHS Hospital or Clinic please let us know so we can send your test results to him or her.

We thank you again for participating in the Strong Heart Study. If you have any questions please call Dr. _____ at the _____ Hospital or Clinic or the medical care provider of your choice. You can also reach me at _____.

Sincerely,

SHS

APPENDIX 15 (b)
Interpretation of Examination Results and Suggestions

Blood Pressure: If your blood pressure was above 140/90, you should go to clinic to see your health care provider for advice on how to lower it.

Percent Body Fat: If your percent body fat is above the desirable range, you should lose weight. The desirable range is shown on the results.

Body Mass Index is also a measure of obesity. If you are more than 120% of body mass index, you should lose weight.

Physical Exam: The findings listed are those found by the exam done by the nurse practitioner. They will be available in your IHS medical record in case you need further evaluation.

Cigarette use: If you smoke, it is advised that you cut down or preferably quit. Smoking causes heart problems and cancer and you can improve your health by quitting.

Lipids: These are the fats measured in your blood.

Total cholesterol measures both good and bad cholesterol.

Triglycerides are another kind of fat in the blood. Sometimes they are high in diabetic patients who have high blood sugars. If your triglycerides are high, eat less food and drink less alcohol (if you drink) and have your triglycerides checked in 3 months.

HDL cholesterol is the good cholesterol. The higher your HDL cholesterol, the less chance of heart disease.

LDL cholesterol is the bad cholesterol which may cause heart disease if it is high: 130-159 is moderately high, over 160 is very high.

All persons with high LDL should go on a low fat, low cholesterol diet and have a follow-up check-up in several months.

Creatinine measures kidney function. If it is high, you should go to clinic to see a health care provider.

Glucose is blood sugar. Patients with diabetes have high blood sugars.
                    Fasting glucose 140 or higher = Diabetes
                    Fasting glucose less than 140 = Normal

Two hour post 75gm glucose load

Some persons were given sugar water to see if they have diabetes. The reading two hours after drinking the sugar means:

> Blood glucose 200 or higher = Diabetes
> Blood glucose 140-199   = Impaired glucose tolerance
> Blood glucose less than 140   =Normal

People with impaired glucose tolerance are more likely to get diabetes than people with normal levels. Healthy diet, losing weight if you are overweight, and exercise are an important way to prevent or control diabetes.

Urinary albumin-creatinine ratio measures kidney function. If above 30, it indicates there may be some kidney damage.

Glycated hemoglobin reflects the blood sugar over the past 6 weeks.

The levels of glycated hemoglobin are interpreted in diabetic patients as follows:

> Poor control   - 9.6% and above
> Fair control   - 7.6% - 9.5%
> Good control - 6.0% - 7.5%
> Normal Value - 5.9% and below

APPENDIX 16
THE STRONG HEART STUDY II

INFECTION CONTROL POLICY

Human Immunodeficiency Virus (HIV) and Hepatitis B

INTRODUCTION:

The virus that causes AIDS is a human retrovirus that has been named HIV (human immuno deficiency virus). The virus primarily infects cells of the T-lymphocyte system, but is also able to infect other cells such as macrophages and those of the central nervous system. The virus destroys the cellular immunity of infected people, leaving them susceptible to a variety of opportunistic diseases.

It has been established that the virus can be transmitted: (1) through sexual contact; (2) through parenteral exposure, including sharing needles and syringes when injecting illicit drugs, transfusion of blood or its components, and infusion of clotting factors concentrates; and (3) through perinatal exposure, probably both transplacental and intrapartum transmission and postpartum transmission.

To date, there is no evidence that the HIV virus can be transmitted by casual social contact, not even among people living in the same house-hold. Recent reports by the CDC suggest that exposure of skin or mucous membranes to contaminated blood may rarely result in transmission of HIV. The magnitude of the risk is not known.

Hepatitis B virus (HBV) is transmitted in ways similar to HIV.

PURPOSE:

To stress the importance of following recommended precautions to prevent exposure to the AIDS and HBV virus.

PREVENTION:

1.   Before initiating work, all bench areas should be cleaned and sanitized daily with a appropriate disinfectant.

2.   All laboratory specimens should be treated as if they were contaminated with either HIV or HBV. Any specimens specifically taken from known AIDS or hepatitis patients should clearly marked as isolation and transported in a leak proof container.

3.   Specimens leaking from their containers should be discarded after requesting a

replacement. In those cases in which the specimen is not replaceable, the outside of the soiled container should be disinfected with either a 1:10 sodium hypochlorite solution (household bleach) or lysol spray and left standing for at least ten minutes before performing any laboratory procedure(s).

4.   Every laboratorian should wear gloves and be dressed in a laboratory gown or uniform when handling and processing specimens. This will minimize the risk of contamination to exposed body parts or street clothing. Gloves should beworn and disposed of in accordance with the "Gloves (Proper Use and Disposal)" policy. Hands and other skin surfaces should be washed thoroughly and immediately after coming into contact with blood or body fluids.

5.   Wear masks, gowns (of aprons), and goggles (or glasses when there is a possibility that blood or body fluids may splash or splatter on you.

6.   All laboratory specimens that must be manipulated before processing (i.e., body fluids to be diluted, caps on tubes of blood to be opened, specimens to be split or transferred, etc.) should be handled cautiously.

7.   Centrifuge carriages should be sanitized daily (or after each use if possible HBVs or AIDS specimen is being centrifuged) with a germicide. After weekly use, centrifuge interiors should be sprayed with an appropriate disinfectant.

8.   To prevent needle stick injuries, needles should never be recapped separated from syringes or otherwise manipulated. Instead, used needles should be place intact into puncture-resistant containers. The same criteria should be applied to used scalpel blades and any other sharp device that may contaminated by a patient.

9.   To prevent transmission of HIV or HBV the platform on the finger prick device (Autoclik, etc.) should be changed between patients.

10.   Reusable devices, such as tissue grinders, pipettes, etc, should be placed into vesicles containing an appropriate germicide prior to being autoclaved and cleaned.

11.   Mouth Pipetting of blood or serum or plasma is forbidden for any clinical laboratory procedure. Mechanical pipetting devices are available and must be routinely used.

12.   All laboratory specimens and disposables should be discarded in biohazard bags and autoclaved prior to final disposition by either incineration or sanitary carting.

13.   Accidental spillage of a specimen should be promptly cleaned up with any of the previously mentioned disinfectants. This solution should be freshly prepared and

kept in its diluted form no longer than one week.

14. If accidental contamination occurs to an exposed area of the skin, wash first with a good liquid antimicrobial detergent soap (i.e., hibiclens, chlorhexidine gluconate, etc.). Rinse well with water, then apply a 1:10 dilution of household bleach or 50% isopropyl or ethyl alcohol. Leave preparation on skin surface for at least one minute before final washing with the liquid soap and water.

15. All work bench areas should be cleaned and sanitized with an appropriate germicidal agent at the end of each work shift.

16. Before workers leave the laboratory, all protective clothing should be removed. In addition, all laboratory personnel should wash their hands and arms with an appropriate germicidal detergent soap (i.e., chlorhexidine gluconate with alcohol).

FIRST AID AFTER CONTAMINATION OR LIKELY CONTAMINATION
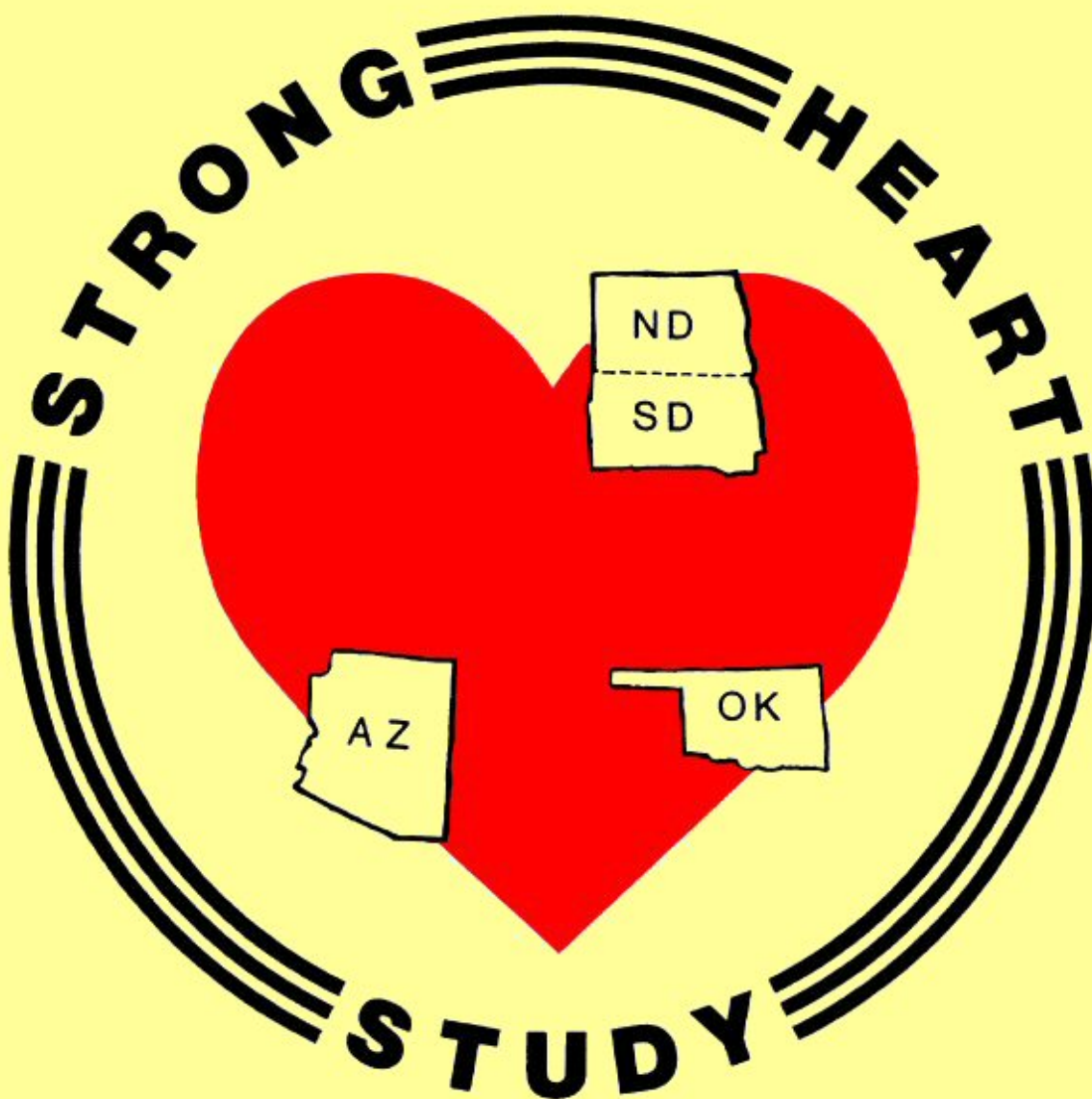
1. SKIN: Wash the skin well with soap and water.

2. EYES: Flush eyes with water by using the safety eye wash.

3. NEEDLE STICK: Squeeze the affected part gently to somewhat cleanse the wound by bleeding. Cleanse with soap and water.

4. MOUTH: Immediately rinse out the mouth with large amounts of clean water. Do not swallow the water. (mouth pipetting is strictly forbidden)

5. For all incidents:

   a. Notify the supervisor and report to the Employee Health Unit or in the event Employee Health is closed, go to the Emergency Room.

   b. As incident report form must be filed.

   c. The decision to administer hepatitis immune globulin is made by the Employee Health Unit.

   d. The hepatitis B surface antigen (HBsag) vaccine HAS BEEN AND IS AVAILABLE to high risk personnel (laboratory, ICU, etc.)

REFERENCE:

Tierno, PM: Preventing Acquisition of Human Immunodeficiency Virus in the Laboratory: Safe Handling of AIDS Specimens. Laboratory Medicine 1986; 11: 696-698.

Standard Procedure for the Collection of Diagnostic Blood Specimens by Venipuncture, National Committee for Clinical Laboratory Standards March 1980.

So You're Going to Collect a Blood Specimen, College of American Pathologist, 1980. Rose SL: Clinical Laboratory Safety Philadelphia, J.B. lippincott Company, 1984

**CARDIOVASCULAR DISEASE IN AMERICAN INDIANS
(PHASE II)**

OPERATIONS MANUAL - VOLUME THREE

LABORATORY PROCEDURES

THE NATIONAL HEART, LUNG AND BLOOD INSTITUTE
OF THE NATIONAL INSTITUTES OF HEALTH

THE STRONG HEART STUDY

Cardiovascular Disease in American Indians
(Phase II)

Operational Manual

Volume Three

Laboratory Procedures

July 1, 1993

For copies, please contact

Strong Heart Study Coordinating Center

Center for Epidemiologic Research

University of Oklahoma Health Sciences Center

P.O. Box 26901
Oklahoma City, OK 73190

MANUAL III

LABORATORY PROCEDURES

LABORATORY PROCEDURES

1.1   Equipment and Supplies

1.   Equipment: A refrigerated clinical centrifuge pre-cooled at 4^0C will be required for the separation of plasma from the cells. The centrifuge rotor should have adapters for 16 x 100 mm, 13 x 100 mm and $10^1/_4$ x 64 mm tubes.

Alternatives would include the placement of a non-refrigerated centrifuge in a standard refrigerator via an extension cord.

2.   Supplies: A description of the various tubes and supplies that will be needed in the study is presented. Except for the Cryovials (and cap inserts) for frozen samples, all other items can be substituted with equivalent items from the local distributor.

Items	Size	Packaging	Material ID
Plastic Tubes w/screw on cap (plasma and urine)	14-ml	1000/case	polypropylene
Cryovials w/screw caps	2-ml	500/case	Corning 25704
White inserts for Cryovial caps		500/case	Corning 25709-W
Blue inserts for Cryovial caps		500/case	Corning 25710-B
Yellow inserts for Cryovial caps		500/case	Corning 25713-Y
Red inserts for Cryovial caps		500/case	Corning 25711-R
Transfer Pipets	7-ml	500/box	polyethylene
Transfer Pipets sterile	7-ml	500/box	polyethylene
Vacutainer, EDTA	10-ml	1000/case	15% solution BD-6457

Vacutainer, SST	9.5-ml	1000/case	BD-6510
Vacutainer, Sodium Citrate	4.5-ml	1000/case	BD-6579
Vacutainer, Fluoride	5-ml	1000/case	BD-6475
Vac. Multiple Sample Needles (Butterfly)	21G	200/case	BD-7251
Vac. Reusable Holder	4 (free with each case of tubes)		

Adhesive tape

[2x2] Sterile Gauze

Alcohol Wipes

Latex Gloves

Tourniquet

Urine collection cups

Bandaids

Needle Disposal Device

===

1.2   Procedure for Blood Drawing

Have the following tubes labelled and ready in an ice bucket

        Fasting Sample
                *Three 10-ml Lavender-top tubes
                *One 5-ml Gray-top tube
                *One 4.5-ml Blue-top tube (citrate)
                *One Special tube
                *One 9.5-ml Red-Top tube (SST)

        2-hr Sample
                *One 5-ml Gray-top tube

        Other Supplies   *Adhesive tape
                *tourniquet   *Vacutainer sleeve
                *alcohol pads   *Vacutainer needle [21G]
                *2x2 gauze pads   (multiple sample)
                *bandaids   *urine collection cups

Note: Participants exempted from the GTT will not have a 2-hr sample collected

NOTE: Gloves must be worn when drawing blood or handling samples. An appropriate barrier must be used at any time there is a risk of aerosols (such as when opening vacutainer tubes.)

1.2.1 One-Touch Procedure

1)   Obtain One-Touch reading from a drop of blood obtained by finger prick. (Using the blood from the venipuncture procedure below will not provide comparable results since there is a difference between capillary blood (fingerstick) and venous blood values.)

2)   See One-Touch procedure for calibrating the meter and steps to follow in obtaining a glucose reading. (Consult with the operations manual which can be obtained from Lifescan, Inc. 1-800-227-8862)

1.2.2 Venipuncture Procedure

1)   Position the participant in comfortable chair in an environment free from distraction.

2)   Query the participant about fasting state. "When was the last time you ate?" Record time since last food or beverage on GTT check list (appendix 6, Volume II). If subject is not fasting, record time and note in comment section what foods or beverage were consumed

that morning. Be sure to include any additives like cream, sugar, or artificial sweeteners if a beverage was consumed. Regardless of fasting state, proceed with drawing procedure.

3) Inform the participant about the procedure. Use left arm if possible.

4) Assemble all materials; have extra tubes within reach.

5) Apply tourniquet; have subject close fist and palpate for vein. (A vein feels like an elastic tube and bounces when pressure is applied). If the presence of vein is questionable, remove or loosen tourniquet. If the structure remains, it probably was not a vein; if it disappears assume it was a vein. Another technique to assist in locating a vein is to moisten the skin with alcohol as it will decrease the friction and may aid in the palpation of a vein. If the tourniquet has been on for 2 minutes, loosen and reapply before performing venipuncture.

6) Cleanse skin over vein thoroughly using a circular motion from center to periphery. Dry with sterile gauze.

   DO NOT TOUCH SKIN AFTER CLEANSING

7) Put gloves on; fit luer adapter needle at end of collection set into Vacutainer sleeve and place lavender top tube into sleeve.

8) Pull skin taut 2 inches below site to keep vein from rolling. With bevel of needle in upright position, enter vein and then push the tube forward as far as it will go. Hold needle in the same direction as vein and at a 15 degree angle to vein.

9) After blood begins to flow, secure butterfly with a piece of tape and loosen the tourniquet.

   If blood does not begin to flow, try the following:

   a) Move the needle slightly in or out.
   b) Rotate needle slightly or lift needle to move bevel away from wall of vein.
   c) Try another tube.
   d) Loosen tourniquet; blood flow may be impeded if tourniquet is too tight.

   * Be sure to watch for signs of hematoma from a vein. If there is any indication of hematoma, immediately remove tourniquet and needle. Place 2x2 gauze over the site, and apply pressure and/or ice pack on site for 5 minutes. If the first attempt to obtain blood is unsuccessful (with the subject's permission) try again on the opposite arm. The same technician should not attempt a venipuncture more than twice.

Proper Venipuncture Angle

15°
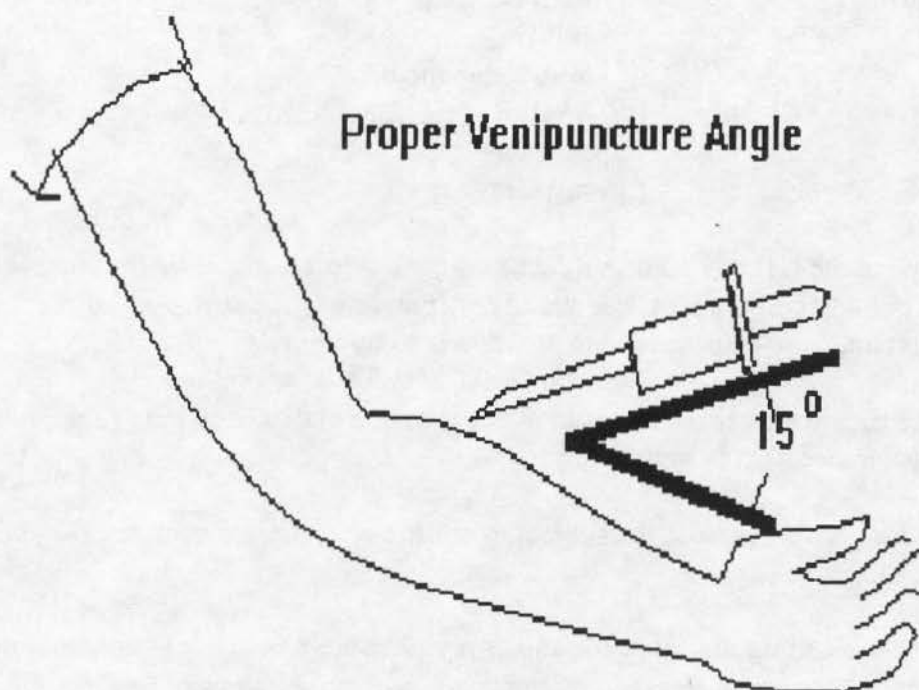
Figure 1. Proper Venipuncture Angle

10)   When first tube is filled, remove tube and replace with the next tube. Invert all filled tubes several times and place on ice.

11)   Proceed with additional tubes in this order:

        Fasting: 3 [10-ml] Lavender top tube
                                        1 [5-ml] Gray top tube
                                        1 [4.5-ml] Blue top tube
                                        1 [9.5-ml] Red top (SST) tube
                                        1 Special tube
        2 Hr: 1 [5-ml] Gray top tube

12)   After drawing the last tube, remove the tourniquet. Place a gauze on the site of the needle entry and quickly withdraw the needle. Apply pressure to the site. Ask subject to hold the arm straight and hold gauze pad with pressure until told to relax.

13)   Record the time the fasting draw is completed on the GTT check list. (See procedure for Glucose Tolerance Test in section 5.6.)

14)   Serve glucose beverage; instruct subject to consume it within 3 minutes. Record time on GTT check list.

15)   Confirm that bleeding has stopped, and apply pressure bandage at venipuncture site. If bleeding has not stopped, elevate arm and continue to apply pressure until it stops.

16)   Affix preprinted labels to tubes, making sure the ID# and tube designation are correct.

17)   Give the participant labeled urine specimen cup and instruct him to void into container. Inform him or her where to leave the container.

18)   Remove gloves, wash hands, and proceed to next participant.

1.3   Sample Preparation and Storage

The laboratory procedures described in this manual are being implemented in the *PENN MEDICAL LABORATORY* (PML) of the Medlantic Research Institute.

1.3.1 General Rules for Handling Samples for Lipids and Other Measurements

One important precaution which should always be kept in mind in handling samples for lipids and lipoprotein measurements is that the blood should be cooled (either in the refrigerator or on ice) as soon as the samples are collected, and kept cold until processing is complete and samples are properly stored. Plasma should be separated from the cells within a few hours. Plasma samples should not be allowed to freeze and thaw during any of the handling steps.

1.3.2 Processing of Blood Samples and Urine Sample

The flow diagram in figure 2 illustrates the blood and urine processing procedure of this protocol. A check list is available in the Appendix 1 and should be completed for each participant. Appendix 2 is a check list for quality controls which will require a number of additional tubes of blood.

1.   Label vials and tubes for each patient as follows (this should be done before beginning the blood processing). Attach the labels according to the labelling diagram in figure 11.3.1. All tubes are to be labelled before processing is begun:

Num/Pat	Container	Label	Storage
1	14-ml Plasma Tube	LIPIDS	refrigerated (4ºC)
5	2-ml Cryovials without inserts	STORAGE	frozen (-70ºC)
2	2-ml Cryovials without inserts	HbA1c	frozen (-70ºC)
1	14-ml Tube	BUFFY CT	frozen (-70ºC)
3	2-ml Cryovial with blue cap insert	COAG	frozen (-70ºC)
3	2-ml Cryovials with green cap inserts	SPECIAL	frozen (-70ºC)
2	2-ml Cryovials with white cap inserts	INSULIN	frozen (-70ºC)
2	14-ml Urine Tube	URINE	frozen (-70ºC)
2*	2-ml Cryovial with yellow cap inserts	0HR GLUC*	frozen (-70ºC)
2**	2-ml Cryovial with red cap inserts	2HR GLUC**	frozen (-70ºC)

* 0Hr Gluc sample will also be used for Plasma Creatinine testing.
** The 2-ml Cryovials with red cap inserts will not be needed for any participant exempt from the GTT

2.   Arrange the vials and tubes on ice in the order in which they are listed in step 2.

3.   Spin all blood tubes at 4°C for 10 minutes, at 1500 RPM (500 x g).

4.   Remove tubes from centrifuge and place them in a rack on ice.

5.   [10-ML] LAVENDER TOP TUBES (total of 3)

    A.   Remove stoppers from all three [10-ml] lavender top tubes.

    B.   With a fresh, disposable transfer pipet, place approximately 6-7 ml into the 14 ml plasma tube. With the same pipet divide all the remaining plasma between 5 [2-ml] Cryovials labelled "Storage." (approx. 1.0 ml each) Be very careful not to disturb the cell layer in the bottom of the Lavender top tubes (leave approximately 0.5 ml of plasma in each tube). Discard pipet after completion of this step.

    C.   Do not freeze the [14-ml] plasma tube, place in refrigerator for weekly shipment. Make sure that the cap has been securely closed. Do not freeze this tube.

    D.   Securely cap and freeze the Cryovials as quickly as possible at -70°C. Once frozen, the samples are to be kept at -70°C until shipment.

    E.   Using a sterile pipet, carefully draw up the buffy coat from the first [10-ml] Lavender top tube and dispense into a prelabelled 14 ml tube labelled "Buffy Ct." The buffy coat is the white layer on top of the red cells. It is rich in white cells. This step is best carried out by positioning the pipet tip slightly above the buffy coat and, while aspirating, carefully moving the tip just over the surface of the buffy coat in a slow swirling motion. The resulting aspirate should contain all of the buffy coat material, plus a small amount of red cells and plasma. When aspirating, try not to let the pipet tip actually touch the buffy coat, or an excess amount of red cells will be drawn up. Similarly, suspending the tip too far above the buffy coat results in too much plasma and too few white cells. This should result in a volume of approximately 1.0-1.5 ml.

    F.   Using a fresh sterile pipet for each tube, repeat step E with the remaining two [10-ml] Lavender top tubes for the same patient, pipetting into the same 14 ml tube.

G.   Immediately after the buffy coats have been dispensed into the 14 ml tube, and the tube <u>securely</u> capped, place the vial on ice, (or immediately into the freezer.) It is important to ensure that the top of the tube is screwed on tightly, otherwise the "O-ring" seal will leak.

H.   The tube which contains the buffy coats is to be frozen at -70°C as quickly as possible. (The specimens are stable if kept on ice for up to a maximum of 4 hours, if necessary, however, *immediate freezing is preferred*.) Once frozen, the samples are to be kept at -70°C until shipment.

I.   Transfer at least 1.5 ml of red cells from the last tube into each of the remaining 2 remaining [2-ml] Cryovials without cap inserts (labelled "HbA1c.") Cap and freeze these tubes as quickly as possible at -70°C.

J.   Replace the stopper in both of the other vacutainer tubes. Label these tubes with the patient ID labels for "Red Cells" and place the tubes in refrigerator for weekly shipment to Memorial Blood Center of Minneapolis (see shipping instructions in section 1.4.) <u>Make sure that the stopper has been securely replaced. Do not freeze these tubes.</u>

K.   Discard the used pipets and the remaining tubes with the red cells.

6.   <u>BLUE TOP TUBE</u>

A.   Remove the stopper from the tube.

B.   With a fresh transfer pipet, divide all of the plasma between the three [2-ml] Cryovials labelled "Coag."

C.   Using a caps with a <u>blue</u> insert, securely cap the vials.

D.   Freeze the vials as quickly as possible at -70°C. Once frozen, the samples are to be kept at -70°C until shipment.

E.   Discard the pipet and tube with the remaining red cells.

7.   <u>SPECIAL TUBE</u>

A.   Remove the stopper from the tube.

B.   With a fresh transfer pipet, divide all of the plasma between the three [2-ml] Cryovials labelled "Special."

C. Using a caps with a <u>green</u> insert, securely cap the vials.

D. Freeze the vials as quickly as possible at -70°C. Once frozen, the samples are to be kept at -70°C until shipment.

E. Discard the pipet and tube with the remaining red cells.

8. RED TOP (SST) TUBE

A. Remove the stopper from the tube.

B. With a fresh transfer pipet, divide the plasma between the two [2-ml] Cryovials labelled "Insulin."

C. Using a caps with <u>white</u> inserts, securely cap the vials.

D. Freeze the vials as quickly as possible at -70°C. Once frozen, the samples are to be kept at -70°C until shipment.

E. Discard the pipet and tube with the remaining red cells.

9. URINE SAMPLE

A. Pour approximately 8 ml of the patient's urine sample into each of 2 tubes labeled "URINE". Discard the remaining sample.

B. Securely cap the tubes and freeze at -70°C.

C. Once frozen, the samples are to be kept at -70°C until shipment.

10. GRAY TOP TUBES

A. Remove the stopper from the fasting [5-ml] gray top tube.

B. With a fresh transfer pipet, divide the plasma between the two [2-ml] Cryovials labelled "0hr Gluc."

C. Using caps with <u>yellow</u> inserts, securely cap the vials.

D. Discard the pipet and tube with the remaining red cells.

E. Remove the stopper from the 2 hour [5-ml] gray top tube.

F.   With a fresh transfer pipet, divide the plasma between the two [2-ml] Cryovials labelled "2hr Gluc."

G.   Using caps with <u>red</u> inserts, securely cap the vials.

H.   Freeze the vials as quickly as possible at -70°C. Once frozen, the samples are to be kept at -70°C until shipment.

E.   Discard the pipets and tubes with the remaining red cells.

1.3.3   Sample storage prior to shipping

Three zip-lock bags will be needed for each participant.

Bag A will be for the refrigerated (4°C) sample to be shipped to Penn Medical Laboratory (Medlantic Research Institute) and should contain the following properly labelled tube:

     * 1 [14-ml] plasma tube ("Lipids")

Bag B will be for the refrigerated samples (4°C) to be shipped to Memorial Blood Center of Minneapolis and should contain the following properly labelled tubes:

     * 2 [10-ml] LTT Vacutainer tube ("Red Cells")

Bag C will be used for frozen samples (-70°C) to be shipped to Penn Medical Laboratory (Medlantic Research Institute) and should contain the following properly labelled tubes:

     *   5 [2-ml] Cryovials for plasma ("Storage")
     *   1 [4-ml] Cryovial for buffy coat ("Buffy Ct")
     *   2 [2-ml] Cryovials for red cells ("HbA1c")
     *   3 [2-ml] Cryovials with blue cap inserts ("Coag")
     *   3 [2-ml] Cryovials with green cap inserts ("Special")
     *   2 [2-ml] Cryovials with white cap inserts ("Insulin")
     *   2 [14-ml] Urine samples ("Urine")
     *   2 [2-ml] Cryovials with yellow cap inserts ("0Hr Gluc")
     *   2 [2-ml] Cryovials with red cap inserts ("2Hr Gluc")

FIGURE 2   **Processing Blood Samples and Urine Sample**

FASTING

| Three [10-ml] Lavender top tubes | One [5-ml] Gray top tube | One [4.5-ml] Blue top tube |

Three [10-ml]
Lavender top tubes

Centrifuge
(1500 RPM, 10 min 4°C)

Plasma   Red Cells

- 1 x [14-ml] tube
  (apx 7 ml)
  DO NOT FREEZE
  ship cold (blue ice)
  ("Lipids" label)

- 5 x [2-ml] Cryovials
  (apx 1.0 ml each)
  (store/ship frozen)
  ("Storage" label)

Remove and
freeze buffy
coats

1 x [4-ml] Cryovial
(store/ship frozen)
("Buffy Ct" label)

Aliquot 2 x [2-ml]
Cryovials for HbA1c
(store/ship frozen)
("HbA1c" labels)

Red Cells for typing
("Red Cells" label)
DO NOT FREEZE

One [5-ml]
Gray top tube

Centrifuge
(1500 RPM, 10 min 4°C)

Plasma (G0)
2 x [2-ml] Cryovials
with yellow cap inserts
(store/ship frozen)
("0Hr Gluc" label)

One [9.5-ml] SST
Red (tiger) tube

Centrifuge
(1500 RPM, 10 min 4°C)

Serum
2 x [2-ml] Cryovials
with White cap inserts
(store/ship frozen)
("Insulin" labels)

One [4.5-ml]
Blue top tube

Centrifuge
(1500 RPM, 10 min 4°C)

Plasma
3 x [2-ml] Cryovials
with blue cap insert
(store/ship frozen)
("Coag" labels)

One Urine
sample

DO NOT
CENTRIFUGE

apx 8 ml
each of
two Urine
transfer
vials
(store and
ship frozen)
("Urine" labels)

2-HOUR

One [3-ml]
Gray top tube

Centrifuge
(1500 RPM, 10 min 4°C)

Plasma (G2) 2 x [2-ml] Cryovials
with yellow cap inserts (store/ship
frozen)   ("2Hr Gluc" labels)

ATTACHING BAR CODED LABELS
TO SAMPLE VIALS

Small Vials (Cryovials)

Attach label with lines in bar
code in horizontal orientation.
(see diagram)

Labelled vial

Large Vials (SC Tubes)

Attach label with lines in bar
code in horizontal orientation.

Allign label with top of vial.
(see diagram)

Labelled vial

FIGURE 3.   ATTACHING BAR CODED LABEL TO SAMPLE VIALS

1.4   Shipping

1.4.1   Shipping Schedule

1.   Refrigerated plasma samples

Refrigerated plasma samples are to be shipped weekly. Ship the samples in approved insulated containers with adequate refrigerant packs to keep the samples cold. DO NOT FREEZE THESE SAMPLES. Samples should not come in direct contact with refrigerated packs.

The samples are to be sent via airfreight, priority overnight delivery, to the following address for receipt by 10:30 AM EST, Monday through Friday. Do not ship on Fridays, weekends, or the day before a holiday:

Penn Medical Laboratory
Medlantic Research Institute
108 Irving Street N.W.
Washington, DC   20010
(202)   877-5481

2.   Refrigerated red cell samples

Refrigerated red cell samples are to be shipped weekly. Ship the samples in approved insulated containers with adequate refrigerant packs to keep the samples cold. DO NOT FREEZE THESE SAMPLES. SAMPLES ARE NOT TO COME INTO DIRECT CONTACT WITH REFRIGERANT PACKS.

The samples are to be sent via airfreight, priority overnight delivery, to the following address for receipt by 10:30 AM EST, Monday through Friday. Do not ship on Fridays, weekends, or the day before a holiday:

Paternity Laboratory
Memorial Blood Center of Minneapolis
2304 Park Avenue South
Minneapolis, Minn   55404
(800)   871-3300

3.   Frozen samples

Frozen samples are to be shipped on dry ice once every 2 weeks. When packing, place at least 8-9 lbs. of dry ice in the box. Pack tightly and do not add any other packing material.

The samples are to be sent via airfreight, priority overnight delivery, to the following address for <u>receipt</u> by 10:30 AM EST, Monday through Friday. Do not ship on Fridays, weekends, or the day before a holiday:

> **Penn Medical Laboratory**
> **Medlantic Research Institute**
> **108 Irving Street N.W.**
> **Washington, DC 20010**
> **(202) 877-5481**

1.4.2 Packing

All samples are to be packed according to DOT regulations and in compliance with shipper's requirements. This includes the following:

* All samples are to be securely caped and sealed in a transport bag.
* Shipping containers are to be self contained with sufficient absorbent material surrounding sample bags to a absorb any spillage.
* The exterior of all packages are to be labelled according to the shipper's requirements.

1.4.3 Shipping Slip

A completed shipping slip form should be put into each shipped container. See appendix 4 for samples of forms. Information required for each participant includes the ID code, the number of plasma tubes, the number of special blood tubes, and the number of blood cell tubes. Place a check mark on the *Frozen Shipment Form* next to the ID number of any participant using insulin. Extra labels are to be mailed along with the bag containing the unfrozen plasma and the whole blood (5 ml PTT) drawn on each patient. These labels will be used by the laboratory.

Put the shipping slip in a plastic bag and place the bag on top of the insulated lid before closing the outside cardboard box.

A copy of the shipping slip should be retained by the originating clinic (Appendix 5).

Upon receipt of the samples by PML, a status check list will be sent back to the PI (or pre-designated individual) by FAX. The condition of the samples received will be noted on the list, along with any discrepancies between the shipping form and samples actually received.

1.4.4   Shipment questions or problems

        If you have any question regarding the status of a shipment contact either Dr. Michael Paidi, Darlene Allen, MT(ASCP), or George Webb at PML. Special shipments for weeks involving a legal holiday are to be coordinated with the laboratory.

                Tel (202) 877-5481
                Fax (202) 877-7342

1.5   Laboratory Procedures

1.5.1   General Laboratory Precautions

1.   All personnel are to wear gloves and protective eye wear whenever handling plasma
       samples. An appropriate barrier must be used whenever there is a risk of aerosols
       (such as when opening tubes.)

2.   Properly dispose of all contaminated supplies and materials.

1.5.2   Plasma Lipids

All lipid determinations will be carried out using the Hitachi 717 Clinical Chemistry
Analyzer using enzymatic kits obtained from Boehringer Mannheim Diagnostics.

1.   CHOLESTEROL ASSAY

PRINCIPLE:

All cholesterol esters present in serum or plasma are split quantitatively (100%) into
free cholesterol and fatty acids by cholesterol esterase:

$$\text{cholesterol esters} \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acids}$$
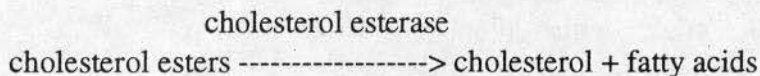
In the presence of oxygen, free cholesterol will be oxidized by cholesterol oxidase
to cholest-4-en-3-one:

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholest-4-en-3-one} + H_2O_2$$
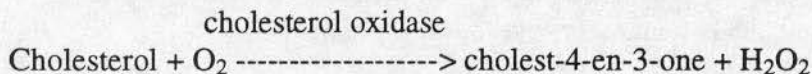
The hydrogen peroxide reacts in the presence of peroxidase (POD) with phenol and
4-aminophenazone to form an α-quinone imine dye:

$$H_2O_2 + \text{phenol} + \text{4-aminophenazone} \xrightarrow{\text{POD}} \alpha\text{-quinone imine dye} + 2H_2O_2$$
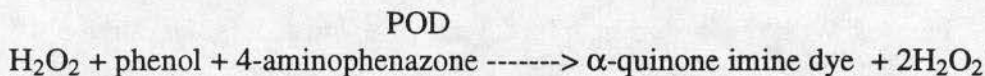
The intensity of the color formed is proportional to the cholesterol concentration and
can be measured photometrically.

SPECIMEN COLLECTION:

Serum: Lipemic samples do not interfere with cholesterol recovery. Hemolysis up to 200 mg/100 ml has no effect on the assay. Bilirubin up to 12 mg/100 ml shows no interference.

Plasma: EDTA is the recommended anticoagulant. Do not use citrate, oxalate or fluoride. Cholesterol is stable in specimens for up to six days at 2-8°C or at room temperature (20-25°C).

REAGENTS:

The Cholesterol Reagent is intended for in vitro diagnostic use. The components of Cholesterol System Pack for HITACHI 717 include:

Cholesterol Reagent

Reactive Ingredients:

0.18 mmoles 4-Aminophenazone
0.73 mmoles 3,4-Dichlorophenol
1.09 mmoles Phenol
$\geq$45 U Cholesterol oxidase (Nocardia erythropolis) (25°C)
$\geq$72 U Cholesterol esterase (microorganism) (25°C)
$\geq$36 U POD (horseradish) (25°)

Nonreactive Ingredients: Buffer, stabilizers

Precautions: DANGER - TOXIC. NEVER PIPETTE BY MOUTH.
In case of contact flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested.

Storage: Store unopened at 2-8°C.

Preparation of Working Reagent:

1.   For R1 Working Solution, pipet 3 ml of Isotridecylpoly(Ethylenglycoether)$_n$ (Genepol) into a 1000 ml volumetric flask. QS with distilled or deionized water to 1000 ml. Mix thoroughly and use this solution as the diluent for the R1 Cholesterol reagent. Reconstitute the contents of one Bottle of Cholesterol Reagent with 100 ml of diluent. R1 Working Solution is stable for four weeks at 2-12°C or 7 days at (20-25°C)

2.   R2 Working Solution is not required

INSTRUMENT SETTING:

CHANNEL SETTING: (see page 7 of Operators' Manual)

CHANNEL NO: 1
TEST CODE: 11

CHEMISTRY PARAMETERS:

TEST CODE:	[Chol]
ASSAY CODE:	[1 Point] : [24] - [0]
SAMPLE VOLUME	[3] [2]
R1 VOLUME	[300] [100] [NO]
R2 VOLUME	[75] [100] [NO]
WAVELENGTH	[700] [505]
CALIB. METHOD	[LINEAR] [0] [0]
STD. (1) CONC.-POS.	[0] - [1]
STD. (2) CONC.-POS.	[156] - [2] (Varies with lot)
STD. (3) CONC.-POS.	[0] - [0]
STD. (4) CONC.-POS.	[0] - [0]
STD. (5) CONC.-POS.	[0] - [0]
STD. (6) CONC.-POS.	[0] - [0]
SD LIMIT	[0.1]
DUPLICATE LIMIT	[100]
SENSITIVITY LIMIT	[1600]
ABS. LIMIT (INC/DEC)	[0] [INCREASE]
PROZONE LIMIT	[0] [LOWER]
EXPECTED VALUE	[0] - [200]
TECH. LIMIT	[0] - [450]
INSTRUMENT FACTOR	[1.00]

CONTROLS:

After every 60 test, there will be six controls analyzed. S_1, S_2 and S_3 are supplied by Northwest Lipid Lab, and PreciCAL, Precitrol-Norm and Precitrol-Abnormal are from BMC.

2.   TRIGLYCERIDE ASSAY

PRINCIPLE:

Free glycerol is eliminated prior to hydrolysis of triglycerides in a preliminary reaction in which lipase in 4-amino phenazone are omitted. This reaction is followed by

enzymatic hydrolysis of triglycerides and determination of liberated glycerol by a fully enzymatic calorimetric assay reaction.

$$GK$$
1.   free glycerol + ATP ------->- 3- phosphate + ADP

$$GPO$$
2.   glycerol - 3 - phosphate + O_2 -------> dihydroxyacetone phosphate + H_2O_2

3. H_2O_2 + 4-chlorophenol -------> oxidation product (does not react with 4-aminophenazone

$$lipase$$
4.   triglycerides + $3H_2O$ -----------> glycerol + fatty acids

$$GK$$
5.   glycerol + ATP ---------> glycerol-3-phosphate + ADP

$$GPO$$
6.   glycerol-3-phosphate + O_2 ---------> dihydroxyacetone phosphate + H_2O_2

$$peroxidase$$
7. H_2O_2 + aminophenazone + chlorophenol ---------------> 4-(p-benzoquinonemo-nimino) phenazone + $2H_2O$ + HCl

SPECIMEN COLLECTION:

Plasma: EDTA will be used as anticoagulant. Triglycerides are stable in plasma for 3 days at 2-8ºC or frozen at -20ºC.

REAGENTS:

The Triglycerides (GB) Reagents are intended for in vitro diagnostic use.

The components of the Triglycerides (GB) System Pack for HITACHI 717 include:

REAGENTS

1.   Buffer

Reactive Ingredients:

        0.15 mol/L Tris buffer, pH 7.6

17.5 mmol/L Magnesium sulfate
10 mmol/L EDTA, disodium salt
3.5 mmol/L-4-Chlorophenol
6 umol/L Potassium hexacyanoferrate (lll)
0.15% Sodium cholate
0.12% Hydroxypolyethoxy-n-alkanes
Nonreactive Ingredient:
Preservative

1a.   Enzymes

Reactive Ingredients:

$\geq$0.05 mmol ATP
$\geq$20 U Glycerol kinase (EC 2.7.1.30; *Candida mycoderma;*25 ^{0}C)
$\geq$250 U Glycerol phosphate oxidase
(EC 1.1.3.21;25 ^{0}C)
$\geq$15 U Peroxidase (EC 1.1.1.7; horseradish; 25 ^{0}C)

2.   Buffer

Reactive Ingredients:

0.15 mol/L Tris buffer, pH 7.6
17.5 mmol/L Magnesium sulfate
10 mmol/L EDTA, disodium salt
3.5 mmol/L 4-Chlorophenol
6 umol/L Potassium hexacyanoferrate (lll)
0.15% Sodium cholate
0.12% Hydroxpolyethoxy-n alkanes
Nonreactive Ingredient
Preservative

2a.   Lipase/4-Aminophenazone

Reactive Ingredients:

$\geq$U Lipase (EC 3.1.1.13; *Pseudomonas species;* 25^{0}C)
0.035 mmol 4-Aminophenazone

Precautions: Exercise the normal precautions required for handling of all laboratory reagents.

Storage: Store tightly closed at 2-8ºC.

Preparation of Working Reagents:

1.   For R1 Working Solution, connect one Bottle 1 (Buffer/Enzymes) to one bottle 1a
        (GPO) using one of the enclosed adapters. Mix by gentle inversion. Completely
        dissolve the lyophilized in the buffer. For R_2 working solution, connect one bottle
        of 2^a to one of 2_b using one of the enclosed adapters. Working Solutions are stable
        for 2 weeks at 1-12ºC or 2 days at room temperature (20-25ºC).

INSTRUMENT SETTINGS:

CALIB. METHOD	[LINEAR] [0] [0]
STD. (1) CONC.-POS.	[0]-[3]
STD. (2) CONC.-POS.	[139]-[2] (Varies with lot)
STD. (3) CONC.-POS.	[0]-[0]
STD. (4) CONC.-POS.	[0]-[0]
STD. (5) CONC.-PDS.	[0]-[0]
STD. (6) CONC.-POS.	[0]-[0]
SD LIMIT	[0.1]
DUPLICATE LIMIT	[100]
SENSITIVITY LIMIT	[0]
ABS. LIMIT (INC/DEC)	[0] [INCREASED]
PROZONE LIMIT	[-32000][LOWER]
EXPECTED VALUE	[0]-[200]
TECH. LIMIT	[30]-[1000]
INSTRUMENT FACTOR	[1.00]

3.   HDL-CHOLESTEROL ASSAY

PRINCIPLE:

        In the presence of $MnCl_2$ and heparin, Chylomicron, VLDL and LDL are
selectively precipitated, leaving only HDL in solution. The precipitated lipoprotein are
sedimented by centrifugation and the clear, HDL-containing supernatant is recovered for
measurement of cholesterol.

HDL-CHOLESTEROL STANDARDS:

        Preciset Cholesterol standard containing 50 mg/dl is obtained from Boehringer
Mannheim Corporation (BMC)

REAGENTS

1.   Analyzer Reagents

        The reagents for testing samples on the Hitachi 717 are the same as the ones used
        for total cholesterol.

2.   Precipitation Reagents

        Manganese Chloride ($MnCl_2$-$4H_2O$, Sigma M-3624)
        Sodium Heparin (20,000 usp units/ml LyphoMed Cat.No.9155-01)

3.   Preparation of Working Precipitation Reagent

        a.   Manganese Chloride ($MnCl_2$-$4H_2O$, Sigma M-3624) should be stored in a
                vacuum desiccator to minimize water uptake; Dissolve 22.2 gm of
                Manganese Chloride in 100 ml of fresh deionized water.

        b.   Working reagent should be made fresh every 2 days using 0.6 ml of
                Sodium Heparin (20,000 usp units/ml LyphoMed Cat.No.9155-01) and 5
                ml of $MnCl_2$ solution.

PRECIPITATION PROTOCOL:

a.   Heparin-Manganese precipitation should be performed as soon as possible after
        sample collection, preferably on the day the samples are drawn. If necessary,
        however, plasma may be stored at 4^0C for no longer than 7 days before the HDL-
        fraction is prepared. In most cases, storage related changes are detectable, but
        small during this time.

b.   Reconstitute one vial of Precinorm-L (PNL) Special Lipid Control Serum (BMC-
        781827) with 3 ml deionized H_2O. Once reconstituted, the sample is stable for 3
        days at $2-8^0C$.

c.   There are six controls included with each set of 60 samples. Four samples are
        precipitated (S1, S2, S# from Solomon Park and PNL from step "b" above) and
        two are tested without being precipitated (S1 and 50 mg/dl Cholesterol Standard
        from BMC.)

d.   Transfer 500 µl of each HDL precipitation control or unknown plasma to
        individually labeled microfuge tube (1.5 ml capacity). Add 50 µl of
        heparin/manganese reagent (see above). Vortex vigorously and allow samples to
        stand at 4^0C overnight. Sediment the precipitate by centrifugation at 7,500 x g for

15 minutes. The supernatant can be decanted into another properly labeled tube. The supernate should be clear. Cloudy supernates are to be respun and, if necessary, filtered prior to testing. The HDL supernate is ready for HDL-cholesterol determination or can be stored at 4⁰C.

CALIBRATION:

a.   Normal saline/EDTA solution is used as a blank.

b.   50 mg/dl Cholesterol Standard is used in calibrator position 4.

c.   Proceed exactly as for Cholesterol determination using the following channel parameters:

CHANNEL SETTING: (see page 7 of Operators' Manual)

CHEMISTRY PARAMETERS:

TEST CODE:	[HDL]
ASSAY CODE:	[1 Point] : [24] - [0]
SAMPLE VOLUME	[20] [20]
R1 VOLUME	[350] [100] [NO]
R2 VOLUME	[90] [100] [NO]
WAVELENGTH	[700] [505]
CALIB. METHOD	[LINEAR] [0] [0]
STD. (1) CONC.-POS.	[0] - [1]
STD. (2) CONC.-POS.	[55] - [2]
STD. (3) CONC.-POS.	[0] - [0]
STD. (4) CONC.-POS.	[0] - [0]
STD. (5) CONC.-POS.	[0] - [0]
STD. (6) CONC.-POS.	[0] - [0]
SD LIMIT	[0.1]
DUPLICATE LIMIT	[100]
SENSITIVITY LIMIT	[1600]
ABS. LIMIT (INC/DEC)	[0] [INCREASE]
PROZONE LIMIT	[0] [LOWER]
EXPECTED VALUE	[35] - [65]
TECH. LIMIT	[25] - [75]
INSTRUMENT FACTOR	[1.00]

4. LDL-CHOLESTEROL

PRINCIPLE AND METHODOLOGY:

Plasma lipoproteins are heterogeneous, and they are classified according to size and density. The largest and lightest particles are chylomicrons and Very Low Density Lipoproteins (VLDLs). Chylomicrons and VLDLs are composed primarily of triglycerides. Cholesterol is contained primarily in the low density (LDL) and high density lipoproteins (HDL).

Beta quantitation is an accurate and efficient technique used to directly measure VLDL composition, and indirectly measure HDL and LDL cholesterol. Unlike beta estimation, the technique is not affected by hypertriglyceridemia or conditions that affect the composition of VLDL, such as poorly controlled diabetes.

Beta quantitation is dependent upon separating VLDL and chylomicrons from LDL and HDL. VLDL and chylomicrons are floated to the top of a solution of density = 1.006 g/ml (saline) by ultracentrifugation (isopycnic centrifugation). The material at the top is assayed for cholesterol and triglyceride and this represents VLDL-TG or VLDL-CHOL (fasting samples have almost no chylomicrons). The bottom material includes both HDL and LDL cholesterol. TG and CHOL are measured in the bottom material. LDL is calculated by subtracting HDL-CHOL (from whole plasma precipitation) from the bottom CHOL value.

SPECIMEN REQUIREMENTS

The specimen should be freshly drawn plasma which has not been frozen. A minimum of 6 ml of sample is required. Specimens which are shipped to the laboratory are to be packed unfrozen on wet ice (or refrigerant pack equivalent.) Ultracentrifugation should be carried out as soon as possible, but samples may be stored at 2-8 ^{0}C for up to 5 days, if necessary.

NORMAL RANGE (FASTING) (NCEP GUIDELINES)

Cholesterol	Desirable	< 200 mg/dl
	High	> 240 mg/dl
Triglycerides	Goal level should be individually determined	
HDL-Cholesterol	Low Male:	< 35 mg/dl
	Female:	< 40 mg/dl
LDL-Cholesterol	Desirable	< 130 mg/dl
	High risk	> 160 mg/dl

PRECAUTIONS

1. Ultracentrifuges can be extremely dangerous if not operated properly. Only trained personnel are to run this equipment.

2. The ultracentrifuge head has a disk its bottom which is read by the optical tachometer. Be careful not to scratch this disk when handling the centrifuge head.

3. Exercise care when using needles. Do not recap used needles.

4. Properly dispose of all contaminated supplies and materials.

5. The blade for the tube slicer is extremely sharp and should be handled with care.

6. All personnel are to wear gloves and protective eye wear whenever handling plasma samples. An appropriate barrier must be used whenever there is a risk of aerosols (such as when opening tubes.)

REAGENTS, EQUIPMENT, AND SUPPLIES

1. Beckman L7-55 Ultracentrifuge with type 50.4 Ti rotor (with extraction tool)

2. Polyallomer Quickseal tubes-Beckman number 344619

3. Tube heat sealer with sealing rings and caps

4. Tube slicer

5. NaCl solution - d = 1.006 g/ml (do not use any other saline solution in this procedure.)

6. Plastic SC tubes with screw caps marked in ml increments (Sarstedt num 62.550.008)

7. 5 ml syringes (with needles)

8. 3 ml syringes (with needles)

9. 3 ml Pipettor with disposable tips

10. Transfer pipets

GENERAL

At sample set up an analyzer worksheet is to be prepared listing samples to be tested. A "T" and "B" space (for "Top" and "Bottom" samples) should follow each laboratory sequence listing on this worksheet. All notations are to be recorded on this worksheet.

SET UP FOR VLDL SPIN

1. Place a test tube rack on a frozen "blue ice" block in a tray. This will ensure samples are kept cold during the set up process.

2. Line up sample tubes to be set up in a the test tube rack.

3. Two SC tubes with the corresponding laboratory sequence number should have been prepared for each sample set during processing. If these are not available (e.g. if this is a repeat sample) label two tubes with the appropriate laboratory sequence number for each sample.

4. Label ultracentrifuge tubes with appropriate laboratory sequence numbers and place them in a test tube rack with the corresponding sample tube. As a second ID, number the samples to be set up 1-44 in order. Write this number on the ultracentrifuge tube in a different location than that of the laboratory sequence number.

5. Remove the plunger from a fresh 5 ml syringe. Remove the cover from the needle and place the needle through the opening in the top of the first ultracentrifuge tube. Repeat this step for each sample to be set.

6. Pipet 3 ml of plasma into the syringe in the corresponding ultracentrifuge tube. Allow the plasma to drain completely into the ultracentrifuge tube. Using a transfer pipet and a small amount of NaCl solution, rinse the inside of the syringe and allow to drain into the tube. Use the plunger to force out any remaining liquid.

7. Remove and properly discard the syringe. Using a separate syringe, carefully layer NaCl solution over the plasma in the ultracentrifuge tube.

8. Place a sealing ring and cap on the tube and seal the tube tightly with the heat sealer. Gently squeeze the sealed tube to check for leaks.

9. Repeat steps 4-6 for each sample to be set.

10. Place the tubes in the rotor sockets, making certain the rotor is evenly balanced. Place a plastic spacer on the top of each tube.

11. Apply a thin layer of vacuum grease to the O-rings on the rotor lid. Seal the rotor and position it properly in the ultracentrifuge and centrifuge for 16 hours at 15°C at 105,000 x g (39,000 rpm).

RECOVERY OF THE LIPOPROTEIN FRACTIONS

1. At the end of the spin period stop the centrifuge WITHOUT USING THE BRAKE.

2. The samples must be removed as soon as possible after the rotor has come to a complete stop.

3. Gently remove the rotor and transfer it to the area where the tubes are sliced, maintaining the rotor in an upright position at all times.

4. Using the extraction tool, remove the spacer and slowly and carefully remove each tube, avoiding any abrupt movement which would disturb the lipoprotein layers.

5. Carefully lubricate the blade path on the tube slicer and assemble the slicer. CAUTION: THE BLADE IS EXTREMELY SHARP AND CARE MUST BE EXERCISED IN HANDLING.

6. Insert the tube into the tube slicer until the bottom edge of the tube is approximately 2.2 cm from the bottom of the tube slicer. Slice the tube with a quick, smooth thrust of the blade. Do not withdraw the blade at this point.

(Top fraction)

7. Using a fresh syringe and needle, insert the needle through the tube near its top. Withdraw the needle and insert it into the top of the tube again, forming a second hole. Be careful not to lose any of the liquid in the tube. Draw the liquid out of the top section of the tube and transfer it to the first corresponding SC tube.

8. Continue to transfer the liquid with the syringe until you have drawn off all the liquid available.

9. Carefully remove the top of the tube from the slicer. Holding the top piece of the tube upside down and using a fresh transfer pipet, rinse the inside of the piece of tube with a small amount of NaCl solution, being careful to loosen any material packed against the inside of the tube. Transfer this wash solution, with the tubes contents, to the same SC tube. With a separate transfer pipet and an additional

small amount of saline, rinse out the top opening of the tube slicer and transfer this wash solution to the same SC tube.

10.   QS the contents of the SC tube to 3 ml with NaCl solution. *The bottom meniscus of the sample should be precisely even with the top of the 3 ml line in the SC tube. Avoid Parallax!* To properly read a meniscus, you must have your eye at the same level as the meniscus.

11.   Tightly cap the SC tube with a screw cap.

12.   Rinse the top opening of the slicer a second time and discard the wash solution.

(Bottom fraction)

13.   Carefully pull back the blade.

14.   Using another transfer pipet, withdraw the contents of the remaining (bottom) portion of the ultracentrifuge tube and transfer the liquid to the second SC tube.

15.   Remove the remaining portion of the ultracentrifuge tube from the slicer and rinse the interior of the tube with a small amount of NaCl solution, being careful to loosen any material which might be packed inside of the tube. Transfer the wash solution, with the contents of the tube, to the second SC tube.

16.   QS the second tube to 5 ml with NaCl solution.

17.   Tightly cap the SC tube with a screw cap.

18.   Repeat steps 6-17 for each sample.

19.   After completing all samples the slicer is to be disassembled and thoroughly cleaned. Be certain to also properly discard all waste materials and clean work area.

TESTING

1.   Thoroughly mix the top and bottom fractions in the SC tubes by gently inverting each sealed tube several (15-20) times.

2.   Measure the total cholesterol and total net triglyceride in the plasma and top and bottom fractions (the two SC tubes) using the regular testing procedure for these analyses. The plasma and two fractions should be measured consecutively on the same analyzer run (i.e. same tray.)

3.   Measure the HDL-Cholesterol on the plasma using the regular testing procedure.

4.   After test results are obtained, perform calculations as listed in the next section.

RESOLUTION OF PROBLEMS ROUTINELY ENCOUNTERED

Problem	Solution
QNS to set up sample	Use a smaller plasma volume (down to a minimum of 1 ml) and CLEARLY INDICATE THE ADJUSTED DILUTIONS on the analyzer worksheet
"Missed" on dilution	Carefully add saline and bring sample volume to next ml increment on tube. CLEARLY INDICATE THE ADJUSTED DILUTION on the analyzer worksheet

CALCULATIONS

Variables:

TD (Top Dilution)
BD (Bottom Dilution)
TFV (Top Fraction Volume, normally 3 ml)
BFV (Bottom Fraction Volume, normally 5 ml)
PV (Plasma Volume used, normally 3 ml)
Ch (Plasma Cholesterol value - analyzer result)
Tg (Plasma Triglyceride value - analyzer result)
HDL (HDL-Cholesterol value - analyzer result)
TFC (Top Fraction Cholesterol analyzer result)
TFT (Top Fraction Triglyceride analyzer result)
BFC (Bottom Fraction Cholesterol analyzer result)
BFT (Bottom Fraction Triglyceride analyzer result)
TCh (Top Cholesterol - calculated)
TTg (Top Triglyceride - calculated)
BCh (Bottom Cholesterol - calculated)
BTg (Bottom Triglyceride - calculated)
Ch-rec (Cholesterol Recovery)
Tg-rec (Triglyceride Recovery)
TCh-adj (Top Cholesterol - Adjusted for recovery)
TTg-adj (Top Triglyceride - Adjusted for recovery)
BCh-adj (Bottom Cholesterol - Adjusted for recovery)
BTg-adj (Bottom Triglyceride - Adjusted for recovery)
LDL (LDL-Cholesterol value - calculated)

Calculations to be performed:

Sample dilutions:

TD   = TFV/PV
         = 3/3 (Normal volumes)
         = 1
BD   = BFV/PV
         = 5/3 (Normal volumes)
         = 1.667

Net values:

TCh   = TD x TFC
TTg   = TD x TFT
BCh   = BD x BFC
BTg   = BD x BFT

Recoveries:

Ch-rec = (TCh + BCh)/Ch
Tg-rec = (TTg + BTg)/Tg

Acceptable recoveries = 0.90 to 1.10 (i.e. 90-110%)

        Samples with recoveries outside of this range must be retested. Samples with low total values for which the recoveries are still outside of this range on retest may be acceptable if approved by supervisor. Samples for which recoveries are not acceptable on retest must be reset.

Adjustments to net values:

TCh-adj = TCh/Ch-rec
TTg-adj = TTg/Tg-rec
BCh-adj = BCh/Ch-rec
BTg-adj = BTg/Tg-rec

LDLc calculation:

LDL = BCh-adj - HDL

5.   QUALITY CONTROLS

A.   OVERVIEW

Penn Medical Laboratory is the Core Laboratory for several multicentered trials, and is presently standardized by the CDC for lipid measurements. Each calendar quarter, PMl is sent blinded samples to measure total cholesterol, triglycerides, and HDL-cholesterol. Quality Control plasma pools have been prepared, standardized and are measured by the Abel-Kendall technique. A precision check (i.e., same sample in each tray 20 times) is run once a week.

B.   CHOLESTEROL AND HDL-CHOLESTEROL

Daily standardization is accomplished using three pooled serums, (Solomon-Park, Inc.). In addition three lyophilized controls (Precical, and Precitrol-N, and Precitrol-A) from BMC are used for total cholesterol and one lyophilized control (Precinorm-L(PNL)) plus a known standard (Precimat 50 mg/dl Cholesterol Standard) are used for HDL (both from BMC). Accuracy and precision of the cholesterol measurements are ensured by participation in several programs including the Lipid Standardization Program administered by the Centers of Disease Control in Atlanta, Georgia and the College of American Pathologists.

C.   TRIGLYCERIDES

The same daily standardization procedure is used for triglyceride as for cholesterol.

Modified Westgard Rules for Triglycerides and Cholesterol Assay

rule	Variation Observed	Action Taken
1_{2s}	one control sample exceeds $\pm$ 2% of the mean	Warning only. Chart and flag.
1_{4s}	one control sample exceeds $\pm$ 4% of the mean	Reject tray. Possible Systematic Error
2_{2s}	two control samples vary from the mean by $\pm$ 2% both in the same direction	Reject tray. Possible Systematic Error
R_{4s}	One control exceeds the mean by 2%, another is $\geq 2\%$	Reject tray. Suspect Random error.
4_{1s}	four consecutive controls all lying on the same side of the mean	Warning. Suspect systematic error.
10_x	10 consecutive controls all falling to one side of the mean (above or below)	Reject tray. Systematic error suspected.

Means and standard deviations for each control pool are determined after at least 10-15 days of assay. For each control pool for each assay, a graph is constructed showing the mean and the two standard deviation +/- limits (Levey-Jennings Plot). Values for each days' control pool are plotted on this graph as a visual means of inspecting for trends within each assay. When the supply of any one control pool is reduced to approximately 3-months' supply, another control pool is constructed and the mean and standard deviation computed a pool can be replaced without a gap.

D.   RECORDING OF ACTIONS TAKEN ON OUT OF LIMIT CONTROLS

1.   Refer to "Action Taken" column in chart above for steps to be taken for out of limit controls.

2.   Record all QC results, even if it is obviously out of limit.

1.5.3 Glucose

PRINCIPLE:

Hexokinase catalyzes the phosphorylation of glucose by ATP:

$$\text{D-glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$
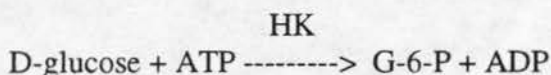
G-6-P is oxidized to 6-phosphogluconate in the presence of NAD by the enzyme glucose-6-phosphate dehydrogenase. No other carbohydrate is oxidized:

$$\text{G-6-P} + \text{NAD} \xrightarrow{\text{G-6-PDH}} \text{gluconate-6 P} + \text{NADH} + \text{H}^+$$
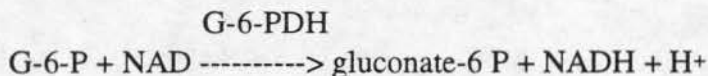
The amount of NADH formed during the reaction is equivalent to the amount of D-glucose in the specimen and can be measured photometrically by the increase in absorbance.

SPECIMEN COLLECTION:

Plasma: Blood must be drawn in gray-topped tubes containing fluoride to inhibit consumption of glucose - by WBC's.

Glucose in plasma with fluoride is stable up to 3 days at 4^0C or for up to 8 hours at 25^0C.

REAGENTS:

The GLUCOSE-HK Reagents are intended for in vitro diagnostic use.

The components of the Glucose-HK System Pack for HITACHI 717 includes:

Non reactive ingredients: 27.7 mmoles Sodium chloride

Reactive Ingredients:

1.2 mmoles Magnesium
433.9 umoles NAD
365.8 umoles ATP
>1130 U Hexokinase (Yeast) (E.C.2.7.1.1)
>951 U G-6-PDH (leuconos.) (E.C.1.1.1.49)

CALCULATION:

The HITACHI 717 analyzer uses absorbance measurements to calculate glucose concentration as follows:

$$Cx = K (Ax - Ab) + Cb$$

Where: Cx = Concentration of sample
K = Concentration factor
AX = Mean of absorbance of Sample + both reagents read at positions 30 and 31 minus mean of absorbance of Sample + the first reagent only read at positions 14 and 15**
Ab = Mean of absorbance of Blank + both reagents read at positions 30 and 31 minus mean of absorbance of Blank + First Reagents read at positions 14 and 15**
Cb = Concentration of Reagent Blank

** Corrected for reagent/sample volume by K =
(sample volume + R1)/(sample volume + R1 + R2).

Linearity: Higher glucose concentrations (350mg/dl) should be reassayed after dilution with physiological saline.

Quality Control: Precitrol - N; Precitrol - Abn; Precical - (calibrator) S_1, S_2, S_3

Procedures for the quality control are the same as those used for cholesterol and triglyceride.

Modified Westgard Rules for Glucose Assay

rule	Variation Observed	Action Taken
1_{2s}	one control sample exceeds $\pm$ 1% of the mean	Warning only. Chart and flag.
1_{4s}	one control sample exceeds $\pm$ 2% of the mean	Reject tray. Possible Systematic Error
2_{2s}	two control samples vary from the mean by $\pm$ 2% both in the same direction	Reject tray. Possible Systematic Error
R_{4s}	One control exceeds the mean by 2%, another is $\geq$ 2%	Reject tray. Suspect Random error.
4_{1s}	four consecutive controls all lying on the same side of the mean	Warning. Suspect Systematic error.
10_x	10 consecutive controls all falling to one side of the mean (above or below)	Reject tray. Systematic error suspected.

1.5.4   Plasma Creatinine (Test performed on 2Hr Gluc sample)

PRINCIPLE:

In an alkaline medium, creatinine forms of a yellow/orange colored complex with picric acid.

                        alkaline
creatinine + picric acid ---------> creatinine-picric acid complex

The color intensity is proportional to the concentration of creatinine present and may be measured photometrically.

Note: Creatinine is stable for 24 hours at 2-8^{0}C. For longer storage, samples must be frozen.

REAGENTS:

The Creatinine Reagents are for *in vitro* diagnostic use only.

WARNING. CORROSIVE. Bottle 1 contains sodium hydroxide. DANGER. TOXIC. Bottle 2 contains picric acid. In case of contact, flush affected areas with copious amounts of water. Get immediate medical attention for eyes or if ingested.

REAGENT PREPARATION:

1   R1 Working Solution:

Use one Bottle 1 (NaOH) as supplied.

2   R2 Working Solution:

Use one Bottle 2 (Picric Acid) as supplied.

REAGENT STORAGE AND STABILITY (after opening):

R1 Working Solution: 4 weeks at 2-12°C.
R2 Working Solution: Until the expiration date at 2-12°C

PREPARATION OF WORKING REAGENTS:

1.   For R1 Working Solution, use contents of one Bottle 1 (NaOH) as supplied. No
     preparation is required. Opened R1 Working Solution is stable at 20-25°C for 4
     weeks.

2.   For R2 Working Solution, use contents of one Bottle 2 (Picric Acid) as supplied.
     No preparation is required. Opened R2 Working Solution is stable at 20-25°C until
     expiration date.

INSTRUMENT SETTINGS:

CHANNEL SETTING: (see Operators' Manual)

CHEMISTRY PARAMETERS: (see Operators' Manual)

TEST	[CREAT]
ASSAY CODE	[RATE-A]:[30]-[35]
SAMPLE VOLUME	[10][5]
R1 VOLUME	[250][100][NO]
R2 VOLUME	[50][100][NO]
WAVELENGTH	[570][505]
CALIB. METHOD	[LINEAR][0][0]
STD. (1) CONC.-POS.	[0.0]-[1]
STD. (2) CONC.-POS.	[***]-[2] (***Varies with lot)
STD. (3) CONC.-POS.	[0]-[0]
STD. (4) CONC.-POS.	[0]-[0]
STD. (5) CONC.-POS.	[0]-[0]

STD. (6) CONC.-POS.	[0]-[0]
SD LIMIT	[0.1]
DUPLICATE LIMIT	[10]
SENSITIVITY LIMIT	[0]
ABS. LIMIT (INC/DEC)	[4500][INCREASE]
PROZONE LIMIT	[0][LOWER]
EXPECTED VALUE	[0.5]-[1.3]
TECH. LIMIT	[0]-[25.5]
INSTRUMENT FACTOR	[1.00]

CALIBRATION:

STD1: Use saline
STD2: Use Precical Calibrator Serum (from Boehringer Mannheim Corp. (BMC))

CALIBRATION FREQUENCY:

Daily

CALCULATION:

The analyzer computer uses absorbance measurements to calculate creatinine concentration as follows:

$$C_x = K(\Delta A_x - \Delta A_b) + C_b$$

Where:

C_x = Concentration of Sample
K = Factor for determining Creatinine concentration, determined by the use of the calibrator
ΔA_x = Change in absorbance per minute of Sample + R1 + R2 read during cycles 30 through 35.
ΔA_b = Change in absorbance per minute of STD 1 + R1 + R2 read during cycles 30 through 35.
C_b = Concentration of STD 1.

LINEARITY: Up to 25.0 mg/dL

EXPECTED VALUES:

Male:	0.6 - 1.1 mg creatinine/dL specimen
Female:	0.5 - 0.9 mg creatinine/dL specimen

QUALITY CONTROL:

Six Quality Control Samples are run after every 60 samples: Three lyophilized samples from BMC (Precical, Precitrol-N, and Precitrol-A) and three frozen samples from Solomon-Park, Inc. (S1, S2, and S3.) Standard Westgard Rules are applied to determine actions taken for out of limit controls. Other quality control procedures are the same as those used for total cholesterol.

REFERENCES:

1.   Schirmeister J, et al: *Dtsch. Med. Wschr.*, 1964;89:1018C.

2.   Tietz N: *Clinical Guide to Laboratory Tests.* Philadelphia, WB Saunders Co, 1983 pp 152,154.

3.   Glick MR, Ryder KW: *Interferographs: Users Guide to Interferences in Clinical Chemistry Instruments*, Indianapolis, Science Enterprises Inc, 1987 and data on file.

4.   Tonks DB: *Canad J Med Tech*, 1967;30:38-54.

Refer to package insert for additional information.

1.5.5   Insulin

PRINCIPLE:

Insulin in serum or plasma is measured by radioimmunoassay. ^{125}I-insulin and nonlabeled insulin from either standards or unknown sera compete for the binding sites on the first antibody (guinea pig anti-porcine insulin serum) during incubation. The bound antigen-antibody complex is then precipitated through the use of a carrier (normal guinea pig serum in PEG buffer) and a second antibody to the carrier (goat anti-guinea pig IgG serum). After centrifugation, the resulting pellet is counted in a gamma counter. Quantitation is achieved by interpolation from a standard curve (Maciel Associates, RIA-PC Version 5.0).

METHODOLOGY:

Total insulin in serum or plasma is measured by radioimmunoassay.

SPECIMEN REQUIREMENTS:

A blood sample is collected by venipuncture in a 5 ml or larger EDTA vacutainer (Lavender top). Insulin can be measured equally well in serum if desired. Place the tube on ice and process as soon as possible. Separate the plasma from the cells by centrifugation at 3,000 rpm for 5 minutes at 4°C. Samples not assayed immediately should be stored frozen at -70°C or lower. Before testing, allow the samples to come to room temperature and mix by gentle inversion. Precaution must be taken when using heparin as an anticoagulant since an excess of heparin will yield falsely high values.

NORMAL RANGE (FASTING):   8 - 15 uU/ml

REAGENTS:

> 0.05 M phosphate saline, pH 7.4
> NaCl 9 g/L
> Na_2HPO_4 (anhydrous) 6.54 g/L
> KH_2PO_4 0.65 g/L
> EDTA 9.3 g/L
> EMTSA 0.1 g/L (ethyl mercurithiosalicylic acid, sodium salt).
>
> Adjust to pH 7.4 with 10 N NaOH

Assay buffer:

> 0.05 M Phosphate saline with 1% BSA. (Bovine albumin, RIA grade, sigma). pH 7.4

Buffer for the second antibody and the carrier (NGPS):

> 0.05 M Phosphate saline with 3% polyethylene glycol (MW 8,000). pH 7.4

Wash buffer:

> 0.05 M phosphate saline without BSA and PEG. pH 7.4

First antibody:

> Guinea pig anti-porcine insulin serum. Obtain from Linco research, Inc. cat no. 1012. Reconstitute with 100 mls of assay buffer. A titration test is recommended for each new lot of first antibody to obtain 60-70% B_0 binding. Aliquot hydrated antibody and store frozen at -20°C or lower.

Linco Research, Inc.
16217 Westwoods Business Park Drive
St. MO 63021
Tel: 314-394-3900
Fax: 314-527-6080

Second antibody:

Goat anti-guinea pig IgG serum. Reconstituted with 100 mls of 3% PEG buffer. Obtain from Linco Research, Inc. Cat No. 2020. Aliquot hydrated antibody and store frozen at -20°C or lower.

Normal guinea pig serum carrier:

Cat no. 38119-3. Aliquot and store frozen at -20°C. Dilute to 1:70 with 3% PEG buffer before use. Obtain from:

Pel Freez
P.O. Box 68
Rogers, Ark 72757

^{125}I-insulin (HPLC purified, monoiodinated A_{14}, receptor grade):

Obtain from Linco Research, Inc. Cat no. 9011. Each vial contains 5 uCi. Reconstituted with 25 ml of assay buffer. Adjust radioactivity to 10,000 cpm/50ul for assay use.

Human insulin standard:

Lyophilized biosynthetic insulin is obtained from Linco research. cat no. 8014. Each vial contains 2,000 uU. Hydrate with 10 mls of assay buffer to produce a 200 uU/ml solution (stock A). Further dilute to prepare 5 ml of each the following standard points:

1. Stock A	200 uU/ml
2. Add 2.5 ml of stock A to 2.5 ml of buffer	100 uU/ml
3. Add 1.25 ml of stock A to 3.75 ml of buffer	50 uU/ml
4. Add 0.5 ml of stock A to 4.5 ml of buffer	20 uU/ml
5. Add 0.25 ml of stock A to 4.75 ml of buffer	10 uU/ml
6. Add 0.12 ml of stock A to 4.88 ml of buffer	5 uU/ml
7. Add 0.05 ml of stock A to 4.95 ml of buffer	2 uU/ml

Aliquot and store at -20°C.

Quality control 1, 2, 3. Cat no. C-370-5, Level I, II, III.

>Bio-Rad
>1000 Alfred Nobel Drive
>Hercules, CA 94547
>Tel: 1-800-227-1600
>Fax: 415-724-5024

Quality control 4, 5, 6. Cat no. 9760. Tri-level Ligand control.

>Ciba-Corning Diagnostic Corp
>63 North Street
>Medfield, MA 02052
>Tel: 508-359-7711
>Fax: 508-359-2879

Quality control 7, 8, 9. Cat no. CON6. Tri-level control.

>Diagnostic Product Corporation
>5700 West 96th Street
>Los Angeles, CA 90045

Reconstituted QCs are stable at 4°C for a week or up to 2 months at -20°C or lower.

<u>All components except the precipitating solutions (second antibody and serum carrier) must be at room temperature before use.</u>

ASSAY PROCEDURE (DAY 1):

1. Label 12 x 75 mm test tubes according to the protocol.

2. Add reagents as follows:

Tube No.	Std	Sample	Buffer	1st Ab	Tracer	Carrier	2nd Ab
1-3 Total	-	-	-	-	50 ul	-	-
4-6 NSB	-	-	200 ul	-	50 ul	100 ul	100 ul
7-9 (0)	100 ul	-	50 ul	50 ul	"	100 ul	100 ul
10-12 (2)	100 ul	-	"	"	"	"	"
13-15 (5)	"	-	"	"	"	"	"
16-18 (10)	"	-	"	"	"	"	"
19-21 (20)	"	-	"	"	"	"	"
22-24 (50)	"	-	"	"	"	"	"

25-27 (100)	"	-	"	"	"	"	"
28-30 (200)	"	-	"	"	"	"	"
31-34 QC1	-	100 ul	"	"	"	"	"
35-38 QC2	-	100 ul	"	"	"	"	"
39-42 QC3	-	"	"	"	"	"	"
43-46 QC4	-	"	"	"	"	"	"
47-50 QC5	-	"	"	"	"	"	"
51-54 QC6	-	"	"	"	"	"	"
55-58 Unkwn	-	"	"	"	"	"	"

3. Pipet 100 ul of the standards, QCs and samples to each test tube. Total, NSB and standards are run in triplicate; QCs, in quadruplicate; patient samples, in duplicate.

4. Pipet 200 ul of assay buffer to the NSB tubes and 50 ul of assay buffer to standards, QCs and the unknowns.

5. Add 50 ul of first antibody to all tubes except the Total and NSB.

6. Add 50 ul of ^{125}I-insulin to all tubes. Vortex for 15 seconds.

7. Cover the racks and incubate at 4°C overnight.

ASSAY PROCEDURE CONTINUED (DAY 2):

8. Add 100 ul of guinea pig serum carrier to all tubes except the total.

9. Add 100 ul of second antibody to all tubes except the total.

10. Vortex, and incubate at 4°C for 2 hours.

11. Add 1 ml of wash buffer to all tubes except the total.

12. Vortex and centrifuge at 3,500 rpm (2,500 X g), 4°C for 30 minutes.

13. Decant the supernatant. Let the tubes stand inverted on absorbent paper. Use cotton balls to remove residual droplets.

14. Using a gamma counter, count the precipitate of each tube for 2 minutes.

CALCULATION OF RESULTS:

The raw count data are reduced by RIA data reduction system (Maciel Associates, RIA-PC Version 5.0).

LIMITATIONS OF PROCEDURE:

1. Assay should be rejected if 3 of 9 reference controls fall outside of the reference range. According to Westgard's rules as modified by Penn Medical Laboratory, controls from each assay are tracked on a Levy-Jennings plot. Results are not released until the assay and QC samples are reviewed by the laboratory supervisor and medical director.

2. If the CV between duplicate results is greater than 15%, rerun the sample.

3. The sensitivity of this assay is 0.23 uU/ml. If sample values greater than 100 uU/ml, dilute the sample with assay buffer and re-run.

1.5.6 Urine Albumin

This assay is performed by the laboratory of Dr. Peter Bennett at the NIDDK Research Center in Phoenix, AZ, using an automated nephelometric immunochemical procedure. Light scattering units are measured during a reaction of albumin with a monospecific antibody to human albumin, using a Behring Nephelometer. Albumin concentrations are calculated by the instrument using a multipoint calibration curve constructed from a commercial calibrator which corresponds to WHO standards. Two normal and two abnormal controls are included in each run. An assay is acceptable if no violations of modified Westgard multi-rules occur. Samples are run in duplicate or triplicate; coefficient of variation for replicates must be less than 7.07. The mean of two values or median of 3 values is reported.

1.5.7 Urine Creatinine

Urine creatinine is measured in the Phoenix NIDDK Laboratory using an kinetic alkaline picrate methodology run on the Ciba-Corning 550 Express Chemistry Analyzer. It is based on a procedure described by Chasson, Grady, and Stanley in 1961. Two normal and two abnormal controls are included at the beginning and end of each run. An assay is acceptable if no violations of modified Westgard Multi-rules occur. Samples are run in duplicate. Coefficient of variation for replicates must be less than 3.54. Mean of duplicates or median of triplicates is reported.

1.5.8 Fibrinogen: Functional Method (BBL Fibrometer)

This assay is contracted to the laboratory of Dr. Russ Tracy at the University of Vermont.

Purpose: The Dade method for determining fibrinogen concentration is based on the clotting time of citrated plasma using 100 NIH units/ml of thrombin (excess thrombin).

Principle: Fibrinogen, a soluble plasma protein, is converted to fibrin, an insoluble polymer of fibrinogen, in the presence of thrombin. Fibrinogen concentration determines the reaction rate using high concentrations of thrombin and relatively low concentrations of fibrinogen. The thrombin clotting time versus fibrinogen concentration is linear when plotted on log-log paper. Therefore, the longer the clotting time, the lest the concentration of fibrinogen.

Equipment:
1. BBL Fibrometer
2. 0.3 ml fibrometer probe
3. 12 x 75 mm plastic tubes
4. 50 ul, 100 ul, 1000 ul MIA pipettes, fibropipet, and P1000 pipet
5. Fibrometer reaction cups
6. Disposable MIA and fibrotip pipette tips
7. 1 ml volumetric pipet

Reagents:

Stock Chemicals

1. Bovine thrombin, topical Parke-Davis 10,000 NIH Units
2. Sodium Diethylbarbiturate (NA-Barbital) $NaC_8H_{11}N_2O_3$ F.W. 206.18 Fisher Scientific B-22 500 gms
3. Sodium chloride (NaCl F.W. 58.44) Sigma
4. Concentrated Hydrochloric Acid (HCL)
5. 85% Phosphoric Acid

Stock Reagents

1. 0.1 N HCL

   Slowly add 50 ml 1M HCL to 250 mls Milli-Q water in a 500 ml volumetric flask. Fill the 500 ml mark with Milli-Q water. Make fresh and use immediately.

2. Veronal Buffer

   Mix together:

11.456 gm Na Barbital
14.610 gm NaCl
43 mls 1N HCL or 430 ml .1N HCL (both freshly made)

Add these reagents to approximately 1000 mls Milli-Q water in a 2000 ml volumetric flask. When dissolved in about 1800 mls of distilled water, adjust pH to 7.35, fill 2000 ml mark and recheck pH. Store at 4^0C. Stability is 6 months.

3. Veronal Buffer with 5mM $CaCl_2$. (for use with EDTA and lavender plasma) Made same as #2 although .734 g.L $CaCl_2$ is also added to the solution.

4. 1% Phosphoric Acid

    Slowly add 11.76 mls of 85% phosphoric acid to approximately 500 mls distilled water in a 1 liter volumetric flask. Fill to 1000 ml mark with distilled water. Store at RT. Stable for one year.

5a. Standard Curve (citrated plasma)

    A standard curve is run daily per technician before assaying samples. All values are read off the daily curve. Dade Fibrinogen Calibration Reference is used by diluting 1 vial with 1.0 Milli-Q water using a volumetric pipet then making dilutions as follows:

    1/5 0.1 ml   standard and 0.4 ml Veronal buffer
    1/15 0.1 ml   standard and 1.4 ml Veronal buffer
    1/40 0.1 ml   standard and 3.9 ml Veronal buffer

    The dilutions are assayed on the fibrometer, in duplicate, and mean values (seconds) are recorded. The standard has an assay value which has been calculated from a previous standard. Each dilution has an assay value of fibrinogen concentration determined by the following:

1/5	Assay value x 2
1/10	Assay value x 1
1/15	Assay value from 1/5 dilution divided by 3
1/40	Assay value divided by 4

For each standard having a specific fibrinogen concentration there is a corresponding thrombin clotting time. Linear regression is used on a Lotus 123 spreadsheet (F7double.wk1) to establish a standard curve using the values obtained. Curves are calculated with every fibrometer run.

5b.   Standard Curve (EDTA plasma) (revised 9/15/89)

For samples drawn into EDTA tubes, a standard curve using plasma treated with EDTA is made. A pool of citrated plasma obtained from 10 donors was determined to have a fibrinogen level of 222 mg/dl in a 1:10 dilution as assayed on the fibrometer. Plasma obtained from an EDTA pool drawn on the same donors was also used. This plasma was run in 1/5, 1/10, 1/15, and 1/40 dilutions. Dilutions were made in Veronal Buffer with 5 mM $CaCl_2$. The dilutions were assayed on the fibrometer in duplicate, and mean values (seconds) were recorded

This procedure was repeated twice, one time each by individual lab techs, MK an DG. Values agreed among the different runs and a standard curve was made according to the above instructions (5a). A standard EDTA curve using 200 NIH U/ml thrombin was also made using the same procedure as above.

5c.   Standard Curve (Lavender plasma) (revised   6/7/90)

For samples drawn into Lavender tubes, a separate standard curve is made. A pool of citrated plasma obtained from 10 donors is determined to have a certain fibrinogen level in a 1/10 dilution as assayed on the fibrometer. Plasma obtained from the same donors was also drawn into a lavender draw tube. This plasma is incubated in the 37^0C water bath in 100 ul aliquots for 1/2 hour and then diluted 1/5, 1/10, 1/15, and 1/40 with Veronal Buffer with 5 mM $CaCl_2$. The dilutions are assayed on the fibrometer in duplicate, and mean values (seconds) were recorded. This procedure is repeated several times by several different lab techs. Average values for each dilution are obtained and a standard curve was made according to the above instructions (5a).

6.   Working Reagents

Thrombin (100 NIH U/ml) PREPARE USING <u>PLASTIC</u> BEAKERS, TUBES, AND PIPETTES. Bovine Thrombin Topical,

Parke-Davis, 100,000 NIH Units - Dissolve each vial of 10,000 NIH Unit thrombin in 100 mls of Veronal Buffer. Make 500 mls, aliquot 4 mls into labelled 5 ml 12 x 75 mm plastic tubes, cap with blue plastic caps, and place into plastic containers with lids. Store at -70°C.

Thrombin (200 NIH U/ml)
Made as above except 50 ml of Veronal Buffer is used to dilute one vial of 10,000 NIH Unit thrombin.

7. Controls/Quality Control

Normal Plasma - Commercial freshly frozen pool (George King Biomedical)
Ci-Trol Level I - lyophilized normal control (Dade)
Abnormal Control - lyophilized low control (Dade)
Red Cross EDTA pooled plasma (Red Cross, Burlington, VT).

Controls are assayed at the beginning of each run, after every 15 to 20 dilutions, and at the end of each run. Controls are assessed on the computer using the standard curve determined for that day on that particular fibrometer. All control values must be plotted on Levy-Jennings graph and all 'out-of-control' values accounted for (i.e., whether a new dilution was made and assayed, whether original dilution was re-assayed, etc.) and reported to the supervisor. Runs are either accepted or rejected based on Westgard Rules (see Quality Control section).

8. Specimen

Recover sample to be assayed from freezer, thaw at 37°C, and place on ice.

Procedure: Turn on fibrometer(s) and heating block(s) to allow for at least 20-minute warm-up time period. Lift the fibrometer probe into position and clean with 1% phosphoric acid. Rinse the electrodes well (x4) with distilled water.

Thaw the necessary number of working thrombin aliquots required for the number of plasma samples being assayed. [i.e., with one 4 ml aliquot approximately 40 cups or 20 samples can be assayed.] After removing aliquots from the freezer, leave at room temperature for 5 minutes, then place into 37°C waterbath until completely thawed. Leave tubes at RT during assays. See discussion #2.

Reconstitute 1 vial of standard material, Ci-Trol Level I, and abnormal control with 1.0 ml Milli-Q water using a volumetric pipet, swirl gently, and allow to equilibrate for 10-15 minutes. Place vial in ice bath with aliquots of thawed pooled plasma and patient's plasmas. Bring the veronal buffer to room temperature.

Dilute pooled plasma, Ci-Trol Level I 1/10 using 0.1 ml sample and 0.9 ml Veronal Buffer. On EDTA plasma, patient samples are diluted 1/20 by pipetting 0.05 ml patient plasma in .95 ml Veronal buffer with 5 mM $CaCl_2$. This dilution was made no earlier than 5 minutes before addition of thrombin in the fibrinogen assay. When samples are run at 1/20 dilutions, some controls must also be run at 1/20.

Lavender plasma patient samples are diluted 1/20 by adding 1.90 ml of Veronal Buffer with 5 mM $CaCl_2$ to the 100 ul of sample that was incubated.

Each dilution is assayed in duplicate. Note: 50 ul of sample and 450 ul buffer may be used for 1/10 dilutions, if necessary.

Pipet 0.2 ml of control and patients' plasma dilutions into Fibrocups at timed intervals and incubate in the fibrometer heating block for at least 2 minutes but less than 5 minutes. Transfer fibrocups, one at a time, to the reaction well.

Pipet 0.1 ml Thrombin (100 NIH U/ml) into the first fibrocup being assayed, at the same time activating the fibrometer's timer.

Pipet 0.1 ml Thrombin (200 NIH U/ml) when running lavender samples and the Red Cross EDTA controls that need to be run with this concentration of thrombin into the first fibrocup being assayed, at the same time activating the fibrometer timer.

Record the number of seconds registered on the fibrometer for each cup, averaging the duplicated and using a mean from which to convert seconds to mg/dl fibrinogen concentration.

If the clotting time is shorter than the lowest value from the standard curve, dilute the EDTA plasma sample 1/30 using 0.1 ml sample and 2.9 ml Veronal Buffer with 5 mM $CaCl_2$ (or dilute the citrate plasma sample 1/20 using 50 ul sample and 950 ul Veronal Buffer). Assay in duplicate. Multiply the calculated fibrinogen concentration by 2 to correct for the 1/20

dilution. Plasma samples having low fibrinogen concentrations, such that no clot forms by the time given for the 1/40 standard (usually 35-40 seconds), are diluted 1/5 using 0.1 ml sample and 0.4 Veronal Buffer, assayed in duplicate. Calculated fibrinogen concentration is divided by 2. (For EDTA samples, dilute 1/10 using 0.1 ml sample and 0.9 Veronal Buffer with 5mM $CaCl_2$.) A dilution less than 1/5 is not usually performed since there is often insufficient volume of plasma sample.

Results: Average the duplicate times for each dilution and calculate the corresponding fibrinogen concentrations from the computer programmed with the standard curve. Report all values in mg/dl, including control values. Make sure all dilutions not 1/10 are corrected by adjusting the correction value in the computer.

Discussion   1.   Fibrinogens are run at 37⁰C.
            2.   The thawed working thrombin is used at RT but placed on ice or refrigerated when not being used.
            3.   Sample dilutions are made soon after samples are thawed so that samples do not sit on ice for a prolonged period.

Reference   Dade Reagents inserts
             Coagulation Lab - MCHV
             Clauss, A. Acta Haemat. 17.237;1957

1.5.9 PAI-I Antigen Assay

PURPOSE: To accurately assess the amount of free PAI-1 in plasmas immunoassay.

PRINCIPLE: A standard two-site ELISA is used, using monoclonal antibody MA-7DA to capture, and monoclonal MA-7F5, conjugated to peroxidase, to generate a signal. This antibody pair is approximately 12-fold more sensitive to free PAI-1 than to PAI -1 in the t-PAI-1 complex, and therefore measures essentially free PAI-1 in plasma.

MATERIALS: 1.   Monoclonal "capture" antibody MA-7d4 (supplied by the Center for Thrombosis and Vascular Research, University of Leuven, Belgium).

                     2.   Monoclonal antibody MA-7F5 conjugated to horseradish peroxidase. (From Leuven)

                     3.   Standard: recombinant PAI-1 (From Leuven)

4.   Control plasma. Prepared by our laboratory.

5.   Costar EIA Plates, # co 3590

6.   Coating buffer: 0.04 M Na_2PO_4, 0.13 M NaCl, pH 7.4

7.   Blocking buffer: 10mg/ml BSA in PBS

8.   Preserving solution: PBS, 1% mannitol, 2% sucrose

9.   Sample dilution buffer: PBS-0.002% Tween-80, 0.1% BSA, 5 mM EDTA

10.   Conjugate buffer: PBS-0.002% Tween-80 and 0.1% BSA, 5 mM EDTA

11.   Washing buffer: PBS-0.002% Tween-80

12.   Citrate buffer: 0.1. M citrate, 0.2 M Na_4, pH 5

13.   Peroxidase substrate: o-phenylenediamine dihydrochloride (OPD)

14.   30% H_2O_2

15.   4 M H_2SO_4

PROCEDURE: 1.   Coat plate at a concentration of 4 ug/ml MA-7D4 in PBS, 200 uL per well. Cover with film and incubate 48 hours at 4^0C.

2.   Empty wells, and add 200 uL/well blocking solution. Cover and incubate two hours at room temperature.

3.   Wash plate two times with PBS if it will be used immediately. Otherwise:

4.   Wash wells once with PBS and then with storing buffer at -20^0C. Wash the thawed plate once with PBS-Tween80.

5.   Thaw standard, patient samples, control plasma at 37^0C, and let stand on ice. Mix thoroughly.

6. Prepare a standard curve, starting at 20 ng/ml, followed by 2-fold serial dilutions for a total of eight standards. Dilute plasmas at 1/10.

7. Add 180 uL of samples, standards and controls, to each well. Run all in duplicate. Cover with film. incubate 18 hours at 4^0C.

8. Wash plate 4 times. Add 170 ul conjugated MA-7F5. Cover with film and incubate for 2 hours at room temperature in the dark.

9. Wash wells 4-5 times, add 150 ul citrate buffer containing 200 ug/ml OPD and 0.003% peroxide. Cover with lid, incubate for 60 min at room temperature in the dark.

10. Stop the reaction with 50 ul/well 4M H_2SO_4.

11. Read absorbance at 490 nm on either computer-controlled plate reader or H-P robot.

CONTROLS/QUALITY CONTROL:

Use pooled normal plasma as well as plasma spiked with the calibrator PAI-1 for controls. All control values must be plotted on Levy-Jennings graph and all 'out of control' values must be accounted for with a written explanation and reported to the supervisor. Run acceptance is based on Westgard Rules.

CALCULATIONS:

All calculations are done using H-P Titer-Calc software, using 4-parameter fit.

REFERENCE:

Measurement of plasminogen activator inhibitor I biologic fluids with a murine monoclonal antibody-based enzyme-linked immunosorbent assay. P. and D. Collen.

1.5.10 Glycated Hemoglobin

$HBA1_c$ is measured in the Phoenix NIDDK laboratory by a variation of the cation exchange method which is adapted to the HPLC. Specially prepared hemolysate is injected into a small glass column fitted with cation exchange resin. A three buffer step gradient

system is used. A spectrophometer detection is used to continuously monitor the absorbance of the elute 410 and 690 nm. Quantitation is accomplished by computing with an electronic integrator the area under the absorbance curve. For about 10% of the samples, where evidence of decomposition is seen, an affinity chromatographic method will be used as a backup.

Quality control is maintained using a normal and abnormal control at the beginning and end of each run. An assay is acceptable if no violations of modified Westgard multi-rules occur. For the Strong Heart Study, in addition, quality control will be maintained by assaying blinded duplicates of 10% of the samples.

Instrument Start-up

1.   Empty waste containers.

2.   Turn all modules on using off/on switch on surge protector.

3.   Check pump and step gradient controller

       a.   Press standby button on controller.

       b.   Press select button on pump until green light indicating flow rate illuminates.

       c.   Set flow rate as indicated for column in use using up or down arrow key.

       d.   Set high pressure

       e.   Press select button until green light indicating pressure illuminates.

4.   Flush system.

       a.   Press "MAN" "3" "ENT" on step gradient controller. Press run on pump.

       b.   Set timer for 25 minutes to flush with buffer 3.

       c.   Monitor pressure to see that it is stable (<5 units change in display).

       d.   Press "MAN' "1" "ENT" on step gradient controller.

5.   Set hemoglobin detector to 0.005.

6.   Check Sampler

a. Press "FUNCTION" "SET SYS".

b. Check table to see that these parameters are correct:

Sample loop size	20
Thermal Module	[ON]
Temperature	8
Filling Speed	1.0
Overfill Vol	40
Flush Volume	1.0
Remote	[OFF]

c. Press "FUNCTION" "SAMPLE TABLE."

d. Enter sample sequence table 3 "1" "ENTER."

e. Visually check sample probe needle for proper alignment and function, dripping should not occur.

f. Check sampler temp on lower portion of screen. It should be $8^0 \pm 2^0C$.

g. Check to see that sample time is 10.0 min.

7. Check column

a. Temperature set at 30^0C.

b. Heater light should flash intermittently.

Sample preparation

1. Controls

a. Reconstitute controls according to instructions in package insert. Store at 2-8^0C for 7 days. Indicate expiration date on vial.

b. Prepare hemolysate of each control (14 days after reconstitution) daily.

1) Prepare a 7 µl in 1.0 ml dilution of glycosylated controls in hemolyzing reagent. Hold in sample tray. Discard at end of day.

2. Calibrator

a. Reconstitute calibrator according to package to insert using 10 ml of reference standard diluent. Mark expiration date on vial (7 days after reconstitution). Store at 2-8°C.

3. Samples

a. Lavender top blood tubes can be stored for 7 days at 2-8°C or up to 90 days at -70°C.

b. 1.) Add 5 µl blood to 1.0 ml hemolyzing reagent. Mix and incubate for 30 minutes at 37°C. Remove from water bath. Centrifuge for 5 minutes at 3000 rpm.

2.) Prepare sample log sheet listing NIH #, date, visit type and time.

3.) Load hemolysates into auto sampler tray.

Calibration

1. Enter date and time into integrator in format shown on screen.

2. Enter value shown on vial of HbA1c reference standard vial.

3. Fill 2 vials with approximately 0.5 ml reference standard and place in positions 1 and 2 on sample tray.

4. Prepare sequence #1 parameter to read

First vial position   1
Last vial position   2
Sample time   10.0

5. Delete other sequence tables

6. Press start on sampler. Make sure controller is in standby mode.

7. After chromatograms appear on graph paper perform the following checks:

a. Review peak labeling. One peak for each A1A, A1B, f, A1C, and AO should be labeled. If any of these is missing or more than one of any fraction is present, consult with laboratory supervisor, review troubleshooting guide or call BioRad for technical assistance.

b. Check retention time (RT). They should be within the following ranges:

   A1A 1.8 ± 0.3
   A1B 2.5 ± 0.3
   f 3.3 ± 0.6
   A1C 4.3 ± 0.4
   AO 5.5 ± 0.3

   If retention times are not within these ranges, consult with laboratory supervisor or call BioRad for technical assistance.

c. Check calibration response factor. 1.1 ± 0.1 is acceptable. If it is not in this range, repeat 3-6. Consult with laboratory supervisor on Bio-Rad Technical Representative at 800-343-2072 if this range cannot be achieved.

8. Load calibrator into slot #3. Run as sample. A1C should be within 0.2 of value shown on bottle. If it is not, repeat steps 4-8. If value is acceptable, proceed to controls.

Controls

1. Load glycated Hb controls level 1 and 2 in sample tray slots 4 and 5. Modify sequence #1 to show 1st vial position =4 and second vial position =5. Press start.

2. Check control values to see that they are within acceptable range. If not repeat controls. Consult with laboratory supervisor or BioRad Technical Representative at 800 342-2072 if either control is out of range.

Sample Assay

1. Load hemolysates in sample tray after calibrators and controls, starting in position #6.

2. If sampler is idle, modify sequence #1 to show beginning and end vial positions. If sampler is operating, create a new sequence table showing beginning and ending vial positions. (Sampler will always start with sequence #1 if it is idle. It will automatically continue onto next sequence when current sequence is complete.

3. Review each chromatogram carefully. Look for incorrectly drawn baselines, Hbf >5.0, or presence of hemoglobin variants such as HbC of HbS. Repeat any questionable results and review with laboratory supervisor. Contact BioRad Technical representative at 800 342-2072 if supervisory assistance is unavailable.

System shutdown

1.　If sampler has been idle for 20 minutes or more, system automatically flushes with buffers 3 and 1. Flush pump with 10 ml deionized water using syringe attached to flush port on top of pump assembly. Turn all modules (except mobile phase conditioner) off using surge protector switch.

2.　If sampler has not been idle for 20 minutes, flush system as follows.

　　a.　Flush with buffer #3 by pressing "MAN" "3" "ENT" on step gradient controller. Set timer for 15 minutes.

　　b.　After 15 minutes flush with buffer #1 by pressing "MAN" "1" "ENT". Set timer for 5 minutes.

　　c.　After 5 minutes press "STBY".

　　d.　Flush pump with 10 ml. change water using syringe attached to flush port on stop of pump assembly.

　　e.　Turn all modules off (except mobile phase conditioner) using surge protector switch.

Recording results

1.　Controls

　　a.　Enter control valves on laboratory QC program.

　　b.　Photocopy chromatograms and place in lab notebook.

2.　References

　　a.　Photocopy chromatograms and place in laboratory notebook.

3.　Patient samples

　　a.　Cut chromatograms into sections of 3 each and photocopy each section. Review each chromatogram to see that baselines are properly drawn. Look for presence of hemoglobin variants or values of HbF >5.0. Consult with laboratory supervisor before reporting any questionable chromatograms.

　　b.　Record results on lab slips and mark copy of chromatograms with a check mark to indicate results were recorded. Present photocopies of calibration,

controls and patient chromatograms to laboratory supervisor for review and coding.

Procedure for Changing Buffer Bottles

Buffer may be changed during run, as long as buffer being changed is not pumping. Step number on stop gradient controller indicates buffer being pumped. (Change buffer #1 during step 2 or 3, etc.). When switching lines do not wipe weight at pickup end of tubing. Discard any buffer remaining in used bottle.

1.5.11 Red Cell Antigens

I.   The following antigens will be tested in duplicate using frozen, pre-filled "V" bottom microtiter trays supplied by GTI (Genetic Testing Institute, Brookfield, WI):

   A.   ABO: A, B and A,B

   B.   Rh: D, C, E, c, e and Cw

   C.   MNSs: M, N, S, s

   D.   Kell: K, k

   E.   Duffy: Fy^a, Fy^b

   F.   Kidd: Jk^a, Jk^b

   G.   Plate control: sample cells and diluent

II. Batch Sheets

   A.   First typing sheets will be tested as follows:

      1.   Use lot numbers WAA-xx and XAC-xx.

      2.   Color coded as follows:

         a.   White for ABO, Rh and MN.

         b.   Green for Ss, Kell, Duffy and Kidd.

   B.   Second typing sheets will be tested as follows:

1. Use lot numbers W<u>B</u>A-xx and X<u>B</u>C-xx.

2. Color coded as follows:

    a. Blue for ABO, Rh and MN.

    b. Pink for Ss, Kell, Duffy and Kidd.

III. Reverse Grouping

A. Tested by technologist using second typing batch sheets.

B. Plasma is tested in "U" bottom microtiter trays with known A, B, and O cells.

C. If an irregular antibody is found,

    1. Identify the antibody.

    2. Use antigen negative cells to repeat reverse grouping.

IV. Interpretation of the most probable phenotype for each system is made by the technologist performing the testing.

V. Comparison of Results

A. A technologist will compare results when both first and second typings are completed.

B. Discrepant results

    1. Repeat typing in the tube using appropriate controls and procedures for the antisera selected.

    2. Record the repeat typing on the bottom of the batch sheet.

C. When the results agree, both technologists will enter their results in the computer.

Reagents

I. 10% Sodium Azide

A. Sodium Azide   50 gm

B.   QS to 500 ml with deionized H_2O

II.   3% Bovine Serum Albumin (BSA)

    A.   30% BSA   10 ml

    B.   Blood Bank Saline   90 ml

    C.   10% Sodium Azide   1 ml

III.   Wash Solution

    A.   30% BSA   16 ml

    B.   10% Sodium Azide   40 ml

    C.   QS to 4 liters with Blood Bank Saline

IV.   Anti-IgG (Anti Human Serum)

    A.   Anti-IgG   25 ml

    B.   3% BSA   75 ml

    C.   Green food coloring to produce light green color.

Quality Control

I.   For every run of four plates (30 samples) or less, a positive and negative control is run with each antiserum on the plate.

    A.   Positive and negative controls are run on separate plates

        1.   Positive controls must show agglutination.

            a.   Failure to stream.

            b.   Cells plastered to the bottom of the well.

        2.   Negative controls and auto controls must stream.

    B.   A list of current control cells is at each work station.

II.   Plate lot numbers, batch numbers and control results are entered each day of testing on the quality control sheets.

III.   Control Cells

A.   Panel cells and commercially prepared O cells arrive weekly from Prenatal Lab.

B.   A1 and B cells are obtained from Donor Processing Lab as needed.

C.   Fresh aliquots of control cells are thrice washed and resuspended to concentrations as follows:

1.   A 0.5% cell suspension standard is prepared by adding 5 drops of commercially prepared O cells to 25 drops of Blood Bank Saline.

2.   A 0.5% concentration of panel cells in Blood Bank Saline.

3.   A 2-4% concentration of A1, B and O cells in Blood Bank Saline.

Testing Procedure (GTI Plate)

I.   Assemble specimen tubes according to computer generated batch sheets, carefully checking numbers on tubes and batch sheets.

II.   Remove appropriate number of microtiter plates from the -70°C freezer. While plates are still frozen, invert each one and quickly remove and discard the cellophane tape cover.

III.   Allow plates to thaw at room temperature for 15-30 minutes or until crystals are no longer visible in the wells. Cells should be plated within 30 minutes of the plates being thawed.

IV.   Prepare specimen cell suspensions

A.   Label 12X75 tubes with :

1.   Specimen number

2.   The row in which the specimen will appear on the microtiter plate.

B.   Using a plastic transfer pipette with a 1 ml draw, transfer appropriate amount of cell to 12x75 tubes to make a 0.5% cell suspension in blood bank

saline. Compare this to the 0.5% cell standard and adjust the specimen suspension if necessary.

    C.   The technologist using the "Second Typing" batch sheets will plate three drops of plasma into the "U" well microtiter plates at this time for reverse ABO typing.

V.   Label microtiter plates with assigned row numbers. Every 30 or fewer specimens require testing of positive and negative controls on separate plates.

VI.   Add one drop of each cell suspension to the appropriate row of wells on the plates.

VII.   Incubate plates at room temperature for 30 minutes by stacking them on top of each other and covering the top plate with a microtiter plate cover.

VIII.   Process WAA or WBA (ABO, Rh and MN) plates.

    A.   Place plates in GLC centrifuge 20 seconds at 2000 RPMs.

    B.   Place plates at 60^0 angle and allow them to stream for approximately 10 minutes.

    C.   Invert the plates and allow them to stream in the opposite direction.

    D.   Invert the plates again just before recording the results.

IX.   Process XAC or XBC (Ss, Kell, Duffy and Kidd) plates.

    A.   Centrifuge plates in GLC centrifuge 20 seconds at 2000 RPMs.

    B.   Decant the supernatant. Using a Jet Pipet set to dispense 0.9 ml, dispense 2 squirts of wash solution into the wells. Use sufficient force to resuspend cell buttons.

    C.   Centrifuge plates in GLC centrifuge 20 seconds at 2000 RPMs.

    D.   Repeat IX.B. and IX.C. twice. Decant the plate to obtain the driest possible button.

    E.   Add one drop of anti-IgG diluted 1:4 in 3% BSA.

    F.   Agitate the plates on Dynatech Minishaker set at maximum speed until all cell buttons are resuspended.

G.   Centrifuge plates in GLC centrifuge 20 seconds at 2000 RPMs

H.   Place microplates at a 60⁰ angle and allow cells to stream.

X.   Process A1, B and O reverse typing

   A.   The technologist doing second typing batch sheets will add A1, B and O cells to "U" bottom reverse typing plates to which specimen plasma has already been added.

   B.   Centrifuge 5-10 seconds at 2000 RPMs.

XI.   Read and record results.

   A.   ABO, Rh and MN

      1.   Do not grade reactions. Record as (+) or (-).

      2.   The auto control must be negative for valid results.

      3.   Streaming indicates a negative result.

      4.   Positive results

         a.   A solid button that slides to the bottom of the well

         b.   Small agglutinates in the stream.

   B.   A1, B and O reverse typing

      1.   Grade results from 1+ to 4+.

      2.   Note any hemolysis.

   C.   Ss, Kell, Duffy and Kidd

      1.   Do not grade reactions. Record as (+) or (-).

      2.   The auto control must be negative for valid results.

      3.   Streaming indicates a negative result.

4.   Positive results (failure to stream)

        a.   A solid button that slides to the bottom of the well.

        b.   Cells plastered to the bottom of the well.

XII.   Interpret most probable phenotype for each system tested (See ANT.500), compare results to other typing and enter results in the computer.

XIII.   Resolve discrepant results.

A.   When both typings are done on the same day, plates are saved in an upright position until comparisons are made. Discrepant results should first be visually checked on both plates to rule out clerical errors. Any changes at this time should be noted and initialed by technologists.

B.   Retyping of discrepant systems should be done by the technologist comparing results. Such testing should be done by tube typing using appropriate controls and procedures for the antisera selected. Results are noted at the bottom of the batch sheets. If the technologist confirms the results of his/her initial microtiter plate testing, the second technologist must retest those systems in the tube using a different antisera.

C.   Unresolved discrepancies must be further investigated by either MBCM Reference Lab or by GTI.

D.   Discrepant results between ABO front and reverse typings should be resolved by tube typing. Reverse typings of children less than 6 months of age that are not resolved or are QNS may be reported as NR.

E.   Any sample that is positive in the control well must be repeated in the tube. Such tests are run along with an auto control. A DAT is also done if all IAT typing results are positive. The DAT must be negative for results to be valid.

XIV.   Potential Sources of Error

A.   Cell suspension that are too heavy may result in decreased sensitivity. Very weak reactions may be missed. Use a standard as described in ANT.400 IV.B.

B.   Two or more drops of cells in wells may result in false negative results.

C.   Loss of antisera potency may result if ABO plates are not plated within 30 minutes of thawing.

D. If plates are too near the light source, the heat produced may have a negative effect on Human anti-M.

E. Results of ABO plates must be recorded immediately after turning the plates a third time. When most cells have streamed to the bottom of the well, positive reactions may be falsely interpreted as negative.

F. Wells that contain too much saline can overflow during the spin into their neighbors causing false negative results. Hold a filled plate at eye level to make sure the meniscus is visible slightly below the top surface of the plate. If a meniscus cannot be seen reduce the wash volume and try again.

G. If the wells are underfilled during the wash or if the washing device is putting out a lot of foam instead of wash, the plates will be inadequately washed and can cause false negative reactions.

H. Cells that are not resuspended during the wash can result in false negative reactions or in wells that seem to have both plastering and streaming. When the wash solution is injected into the plate, it should be at an angle and force to completely resuspend the buttons. Check the quality of the wash by adding one drop of Coombs' control cells (.05% suspension) to each well, mix the plate on a plate shaker and respin. There should be plastering of the Coombs' control cells on the bottom of the well.

I. If the wash is interrupted or delayed for a period of time, antibody can elute from the cells and result in a false negative reaction.

J. Fibrin or debris in wells may look like agglutinates. In an anti-IgG plate, these bits falling out of a cell button may look like a stream. Remove any fibrin or debris from wells using a wooden applicator stick.

K. Cell drops hanging on to the sides of wells may result in false negative results due to lack of incubation with the antisera. If a drop clings to the side, it can be gently tapped to bring it down or collected with a pipet and replace into the well.

L. The plate can be jarred when the cellophane tape is removed if the plate is thawed and the tape sticks firmly. Remove the cellophane tape while the plate is frozen. If the plate is jarred while sitting on the counter or on the reading rack, the antisera can splash out or splash into other wells. If this occurs, start over. If a plate is jarred while slipping it into a centrifuge carrier, a false positive or false negative reaction can result. If this occurs, start over.

M.	Plates that are not spun hard enough do not make hard, tight cell buttons and the reactions look weak. Plates that are spun too hard may take longer to stream. Plates should be spun for 20 second at 2000 RPMs.

N.	If the pipet used to add cells touches the surface of the plate of the antiserum in the well, it can carry enough antibody to the next well to contaminate it causing false positive results. The pipet should never touch either the microplate or the contents of the wells.

O.	If the cells are not completely resuspended after the anti-IgG is added, the trapped cells will not be exposed to the antibody. These cells will then stream when the plate is put on the rack possibly resulting in a false negative reaction.

P.	If the auto control is positive on either the ABO plate, the IgG plate or both, none of the reactions on that plate are valid. A DAT and an auto control must be run in the tube along with tube typing of the antigens. The auto control well should always be read and recorded first. This well tells the reader how a negative test is reading. When the tails are very short, the reader should examine all typings with extreme caution.

Additional Testing Procedures

I.	Verification Testing

A.	Verification testing is performed on all new samples received from a previously tested individual. ABO, Rh Gci and PGMi typing is performed. Current results are compared to previously reported results by the computer.

B.	Procedure for antigen verification testing.

1.	Generate worksheets for typing. See GRP.300.

2.	Prepare cell suspension and test samples for ABO and Rh systems by tube typing.

3.	Occasionally a sample will previously have been typed for A1. The computer "beeps" during entry in this situation. Look up the case to determine if this is the reason. If this is the reason, "SET" results to A.

4.	Occasionally a sample will have been typed as C^w positive. If the computer "beeps" when entering the Rh type, check this possibility.

C.	Interpretation

See ABO and Rh section of ANT.500 to interpret these phenotypes.

II.   Direct Antiglobulin Testing (DAT)

A.   A direct antiglobulin test detects red cells coated with antibody in vivo. It is performed in paternity testing when a sample appears to have all antigens that are tested by an indirect antiglobulin procedure (i.e.: Ss, Kk, Fy a and b and Jk a and b) or at the request of the director.

B.   Procedure

1.   Prepare a 2-4% cell suspension in saline.

2.   Add 1-2 drops of prepared cell suspension to a 10X75 tube.

3.   Wash the cell at least three times with saline.

4.   Add 2 drops of IgG AHG serum to the tube.

5.   Mix gently and centrifuge 15-20 seconds at 3400 RPMs.

6.   Read macroscopically and microscopically. Record results on batch sheet.

C.   Interpretation

1.   Sample is agglutinated: positive DAT

2.   Sample is not agglutinated: negative DAT

3.   If a sample has a positive DAT none of the indirect antiglobulin tests are reported. An "NR" is entered in the computer for those systems and a coded explanation selected (See GRP.400).

4.   If the sample has a negative DAT, results are accepted as valid after reviewing for the possibility that the sample is from a person with a recent transfusion.

II.   Antibody Panel

A.   An antibody panel is performed in the following situations:

1.   When the pooled O cells used in the reverse typing are reactive.

2.   When there is an apparent typing discrepancy between the forward and reverse typing.

3.   At the request of the director.

B.   Procedure

1.   Label ten 10X75 tubes 1-9 and auto. (A1, A2, B and cord cells may be needed is it appears the antibody may be anti-A1 or IH)

2.   Place 2-3 drops of serum or plasma form the paternity sample in each tube.

3.   Using the panel cells, add 1 drop of the appropriate panel cell to the corresponding tube.

4.   Centrifuge at 3400 RPMs for 10-15 seconds. Read, grade and record results on additional testing panel sheet.

5.   Incubate at RT for 10-20 minutes if necessary. Repeat #4.

6.   If necessary, incubate at 4^0C for 5-10 minutes. Repeat #4

C.   Interpretation

1.   Compare pattern of agglutinated and unagglutinated cell with the posted MBCM panel worksheet and identify antibody.

2.   Obtain reverse cells that lack the antigen to which the antibody is directed and repeat reverse typing to obtain valid results.

3.   If the panel results are inconclusive, contact supervisor for further instructions.

4.   If there isn't adequate serum or plasma in the paternity sample to complete panel testing, no valid ABO typing can be obtained. Enter an "NR" in the computer and select a coded comment to explain (See GRP.400).

Interpretation of Phenotypes

SYSTEM   PHENOTYPE REACTION OF RBC'S WITH:
[--- Unable To Translate ** ---]

ABO		anti-A	anti-B	anti-A,B
	A	+	-	+
	B	-	+	+
	AB	+	+	+
	O	-	-	-

REACTION OF SERUM WITH:

		A$_1$ cells	B cells	O cells
	A	-	+	-
	B	+	-	-
	AB	-	-	-
	O	+	+	-

SYSTEM   PHENOTYPE REACTION OF RBC`S WITH:
[--- Unable To Translate ** ---]

		anti-D	anti-C	Anti-E	anti-c	anti-e
RH	Ror* Ro	+	-	-	+	+
	R1r* R1Ro Ror'	+	+	-	+	+
	R1* R1r'	+	+	-	-	+
	R2r* R2Ro Ror"	+	-	+	+	+
	R2* R2r"	+	-	+	+	-

SYSTEM   PHENOTYPE   REACTION OF RBC'S WITH:
[--- Unable To Translate ** ---]

	Anti-D	Anti-C	Anti-E	Anti-c	Anti-e
Rh (con't) R1R2*					
R1r"					
R2r'	+	+	+	+	+
Rzr					
RzRo					
Rory					
RzR1*					
Rzr'	+	+	+	-	+
R1ry					
RzR2*					
Rzr"	+	+	+	+	-
R2ry					
Rz*	+	+	+	-	-
Rzry					
r*	-	-	-	+	+
r'r*	-	+	-	+	+
r'*	-	+	-	-	+
r"r*	-	-	+	+	+
r"*	-	-	+	+	-
r'r"*	-	+	+	+	+
rry					
ryr'*	-	+	+	-	+
rr"*	-	+	+	+	-
ry*	-	+	+	-	-

* Denotes most probable phenotype
Note: Rh is written as most probable phenotype.

SYSTEM   PHENOTYPE REACTION OF RBC'S WITH:
[--- Unable To Translate ** ---]

		anti-M	anti-N	anti-S	anti-s
MNSs	MNSs	+	+	+	+
	MNS	+	+	+	-
	MNs	+	+	-	+
	MSs	+	-	+	+
	MS	+	-	+	-
	Ms	+	-	-	+
	NSs	-	+	+	+
	NS	-	+	+	-
	Ns	-	+	-	+

SYSTEM   PHENOTYPE REACTION OF RBC`s WITH:
[--- Unable To Translate ** ---]

		anti-K	Anti-k
Kell	Kk	+	+
	k	-	+
	K	+	-

SYSTEM   PHENOTYPE REACTION OF RBC'S WITH:
[--- Unable To Translate ** ---]

		anti-Fya	anti-Fyb
Duffy	Fyab	+	+
	Fya	+	-
	Fyb	-	+
	Fyo	-	-

SYSTEM   PHENOTYPE REACTION OF RBC'S WITH:
[--- Unable To Translate ** ---]

		anti-Jka	anti Jkb
Kidd	Jkab	+	+
	Jka	+	-
	Jkb	-	+

1.5.12 GM Allotyping

I.   GM/KM Routine Testing

   A.   Typing is done on selected paternity samples. The following factors will be tested:

   1.   Gm a, x, g, f, b
   2.   Km 1

   B.   A corresponding antibody screen is also performed in each sample tested.

   C.   Tests will be performed in duplicate in flexible V-bottom microtiter plates using reagents from two separate sources. Agglutination patterns are read by streaming as discussed in ANT.500.

   D.   Worksheets will be printed through the computer generated program (See GRP.300). Each batch consists of 14 specimen numbers and 2 controls.

   E.   Serum or plasma used for testing is obtained from the batch racks or the original sample tubes. If less than 0.3 ml of sample is available, "QNS" the GM and Km testing until other testing is completed.

   F.   Prepare and label samples, controls, reagents and plates as described below.

   G.   See Quality Control section for QC procedures. The GM/Km control plate must be tested and read before or concurrently with the client samples being tested that day. Results must agree with the expected types.

   H.   Results of GM/Km typing using reagents from separate sources (duplicate testing) must agree to be considered valid results.

I.   Children under 6 months of age will not be reported. Testing will be done to find paternal antigens per Dr. Polesky.

II.   Repeat testing is done once to resolve discrepancies.

III. Interpretation

      The technologist performing the tests makes the interpretation of the most probable haplotype.

IV.   Worksheets

      Worksheets are placed in review slot after comparison and computer entry.

Sample and Reagent Preparation

I.   Preparation of coated red blood cells (0.2%)

      A.   An ACD tube of O Rh+ red cells is obtained every 2-3 weeks from a continuously available donor to use in the preparation of coated cells.

      B.   Bottles of undiluted coat reagent are kept in the freezer. Reconstitute lyophilized reagent with distilled water before use.

      C.   Label 1 12 x 75 tube for each coat to be prepared.

      D.   Place 0.1 ml coat plus 0.1 ml irrigation saline in the appropriately labeled tube.

      E.   In a 12 x 75 tube, wash approximately 0.5 ml of reagent O Rh+ cells 3 times in saline. Centrifuge the last wash for 3 minutes and remove all supernatant.

      F.   Add 25 ul packed red cells to each diluted coat.

      G.   Incubate cells and coats at 37°C for 60-90 minutes.

      H.   Wash coated cells 5 times with cold LISS. Centrifuge the last wash for 3 minutes and remove ALL supernatant.

      I.   Label one 10 ml clot tube for each coat prepared. Place 10 ml of cold irrigation saline in each tube.

J.   Make cell suspension by adding 20 ul of the appropriate washed packed cells to the corresponding labeled 10 ml tube of cold saline.

II.   Preparation of agglutinators

A.   Label one 10 ml clot tube for each agglutinator being tested.

B.   Make the selected dilution of each agglutinator using 0.3% albumin in irrigation saline, using appropriate dilutions as specified by in-house titters.

III.   Labeling and preparation of samples to be tested.

A.   Labeling microtiter plate

1.   Seven samples and one 0.3% albumin control are typed on each microtiter plate.

2.   Each plate is labeled numerically.

3.   The samples are typed and screened in horizontal rows of 12 wells labeled alphabetically A through G. The last row on the plate is an agglutination and albumin control row. See example below.

```
       Type                  Screen
1      a  x  g  f  b₀  Km1  a  x  g  f  b₀  Km1
A
B
C
D
E
F
G
```

B.   Label 10 x 75 tubes for each sample to be tested to correspond with the plate and row it will be tested (1A, 1B, 1C, etc.) This is also noted on the batch sheet.

C.   Sample preparation:

1.   For each adult being tested prepare an approximate 1:20 dilution as follows:

a.   Place 100 ul of serum or plasma in an appropriately labeled 10 x 75 tube.

        b.   Add 1.9 ml of irrigation saline.

2.   For each child being tested prepare a 1:10 dilution as follows:

        a.   Place 100 ul of serum or plasma in an appropriately labeled 10 x 75 tube.

        b.   Add 0.9 ml of irrigation saline.

3.   Heat this dilution for exactly 10 minutes at 65°C.

4.   Centrifuge all samples for 3 minutes at 3200 rpms.

5.   Samples that do not become clear due to fat or other particulate matter can be ultra-centrifuged in the HLA lab. Contact HLA personnel for instructions.

Reverse Hemagglutination Technique for GM/KM Typing

I.   Technique

A.   Plate all reagents and specimens after placing the labeled microtiter plates on cold, damp towels.

B.   For each horizontal row of 12 wells, add 1 drop of agglutinator as follows:

      1.   To wells in row 1, add 1 drop of agglutinator Gm(a)

      2.   To wells in row 2, add 1 drop of agglutinator Gm(x)

      3.   To wells in row 3, add 1 drop of agglutinator Gm(g)

      4.   To wells in row 4, add 1 drop of agglutinator Gm(f)

      5.   To wells in row 5, add 1 drop of agglutinator Gm(b)

      6.   To wells in row 6, add 1 drop of agglutinator Km(1)

C.   To wells in rows 7-12, add 1 drop of 0.3% albumin

D.   Using disposable glass calibrated pipettes, place 1 drop of diluted samples in all 12 wells of the appropriate plate and row. Example: Sample labeled 1A is placed in the first plate, row "A."

E.	Place 1 drop of 0.3% albumin in each of the 12 wells in last row.

F.	For each row, add 1 drop of 0.2% coated cells as follows:

1.	To wells in rows 1 and 7, add 1 drop of Gm(a) coated cells.

2.	To wells in rows 2 and 8, add 1 drop of Gm(x) coated cells.

3.	To wells in rows 3 and 9, add 1 drop of Gm(g) coated cells.

4.	To wells in rows 4 and 10, add 1 drop of Gm(f) coated cells.

5.	To wells in rows 5 and 11, add 1 drop of Gm(b) coated cells.

6.	To wells in rows 6 and 12, add 1 drop of Km(1) coated cells.

G.	Place at 18-20°C (cold box) for a minimum of 60 minutes.

H.	Centrifuge plates in Sorval GLC 15-20 seconds at 2200 to 2400 rpms.

I.	Allow plates to drip on 60 degree slant board at 4°C (walk-in) for 5 to 10 minutes.

J.	Allow plates to drip at RT on 60°C slant board for 5 to 10 minutes.

II.	Reading Results/Repeat Testing

A.	Reactions Observed

1.	Unagglutinated cells form a stream within 10 - 15 minutes indicating a negative reaction.

2.	Agglutinated cells remain as buttons, indicating a positive reaction.

B.	Agglutination is read and recorded as visualized.

1.	Wells 1-6 are actual typing results.

2.	Wells 7-12 are the corresponding antibody screen.

a.	Wells 7-12 are recorded "neg" as a group or

b.	Any positive reaction is noted.

C. Any sample which contains agglutination in wells 7-12 does not have a valid allotype result.

D. If results of first and second typing do not agree the test is repeated once.

E. Interpretation is performed and results are entered in the computer.

Interpretation of Phenotypes

I. Results in hemagglutination inhibition system

A. Tests showing no agglutination indicate the presence of that Gm or Km factor.

B. Tests showing agglutination indicate the absence of that Gm or Km factor.

C. Haplotypes are interpreted from the results of testing and recorded on the batch sheet.

D. Common Gm haplotypes include ag, axg, fb, and ab.

II. When no interpretation can be made, no haplotype can be reported. This is indicated by "**". The following explanation may be used on the batch worksheets to explain why no result will be reported (a similar explanation must be made by code when entering the results in to the computer):

A. Unable to neutralize antibody.

B. Unable to confirm duplicate testing

C. Child too young (less than 6 months)

D. QNS for further testing

E. Other: Give explanation

III. Gm factors are inherited as a set of haplotypes which vary according to race.

A. Determination of exclusion is based on inheritance of these haplotypes.

B. If there appears to be an exclusion of maternity or paternity all possible haplotypes must be considered.

Quality Control

I. Quality Control Test Plate

A. Positive and negative controls are run each day of testing.

1. Should include the following types:

a. Gm: axgfb, fb, axg, agfb

b. Km(1): positive and negative

2. Controls are obtained from in-house samples, previously typed samples and commercially prepared controls. A chart of currently used control samples and corresponding results is posted in the Quality Control notebook.

B. A quality control plate is done each day of testing before or concurrently with client samples.

1. Using the reagents prepared for testing on that day.

2. Done exactly as the client samples.

3. Seven known samples are tested as well an albumin and agglutinator control done on the last row of the plate.

a. For each known sample, wells 1-6 is the typing and should correspond to the known type; wells 1-7 should stream.

b. Agglutinator control, wells 1-6 of last row, should show agglutination.

c. Albumin control, wells 7-12 of last row, should stream.

II. The Quality Control record must have the following recorded for each day of testing

A. Initials of technologist and date tested.

B. Reagents source, lot number and dilution used.

C. List of batches tested or specific specimen numbers.

D. Results of the quality control test plate described above.

E.   Quality Control forms are placed in the GM/KM Quality Control notebook.

Addendum to Procedures for Strong Heart Study

I.   All samples arrived at Memorial Blood Center of Minneapolis diluted and in a frozen state.

II.   Samples were tested once, not in duplicate as is our normal protocol.

III.   Allotypes b_1 and Km3 were tested using same procedure as a, x, g, f, b_0 and Km1. Haplotype combinations of ab_0 were tested for b_1 and all Km1 positive samples were tested for Km3.

1.5.13 Leucocyte DNA Isolations

REAGENT PREPARATION

Stock Solution:

1.   Autoclave DI-H_2O (10 x 500 ml) $\rightarrow$ for Reagent preparation

2.   Buffer B: 3.85 g NH_4Cl in 500 ml DI-H_2O (from #1)

3.   Buffer C: 0.198 g NH_4HCO_3 in 500 ml DI-H_2O (from #1)

4.   Proteinase K comes in 100 mg lyophilized powder (AMRESCO: Cat. #0706)

      -    Add 10 ml DI-H_2O (from #1) into the vial to make 10 mg/ml Proteinase K working reagent

      -    Filter this 10 mg/ml Proteinase K

      -    Aliquot this Proteinase-K (10 mg/ml) into 5 ml portion and freeze at -70^{0}C.

5.   Ethanol

6.   Tris saturated phenol (AMRESCO Cat. #0945)

7.   2M Tris pH 7.51 (MW = 121.1); 60.55 g Tris/500 ml DI-H_2O$\rightarrow$ Adjust pH to 7.51

8.   0.2M Tris pH 7.51 $\rightarrow$ 1/10 dilution of #7

9.   0.5M EDTA pH 7.98 (MW = 372.2); 93.05 g/500 ml $\rightarrow$ Adjust pH to 7.98

10. 3M NaOAc pH 5.21 (MW = 82.03); 123.05 g/500 ml → Adjust pH to 5.21

11. TE Buffer: 500 λ 2M Tris pH 7.51 + 200 λ 0.5 m EDTA pH 7.98 in 100 ml H$_2$O

12. TES Buffer

       2.5 ml 2M Tris pH 7.51
       1 ml 0.5 EDTA pH 7.98
       in 500 ml DI-H$_2$O 2.5 g SDS

13. Phenol saturated with TES Buffer: (Wear gloves, and lab coat and work under fume hood) Pour 400 ml of tris saturated phenol in a 100 ml beaker. Add 500 ml of TES buffer into the beaker. Star at room temperature (inside a fume hood) overnight. The next day pour the mixture into a 1000 ml separatory funnel. Let it stand for 1 hour until two separate layers are visible. The top layer is TES and the bottom layer is phenol saturated with TES. Collect the final working phenol solution (bottom layer) into another reagent dispenser bottle. This phenol saturated with TES is stable for 3 months if stored at 4ºC.

Pour the top layer (TES) into a 4 gallon hazardous waste disposal bottle.

14. Working Buffer B & C: 500 ml buffer + 50 ml Buffer C Prepare at least 2 liters.

WORKING PROTOCOL FOR LEUCOCYTE DNA PREPARATION

DAY 1 (wear gloves and lab coat)

1. Leucocytes in 4ml tubes will arrive at Penn Medical Lab. Keep this frozen until processing time. Save SHS labels.

2. Log in samples in SHS log book. There will be a lab number and an SHS number. Save SHS labels.

3. Thaw 4 ml tubes by leaving it at room temperature. Label 50 ml conicals with lab number. Label both the tube and cap.

4. Squirt buffer B & C into each 4 ml tube. Cap and invert it 2 to 3 times and pour it into its corresponding conical tube.

5. Q.S. each conical to 45 ml with B & C.

6. Cap tubes and shake vigorously.

7.   Centrifuge these tubes at 3000 rpm @ 10 min.; 4°C. (1st wash).

8.   Discard upper liquid leaving compacted cells at the bottom. Be careful not to discard the cells at the bottom.

9.   Q.S. Conical to 20 ml with B & C.

10.   Centrifuge again at 3000 rpm @ 10 min.; 4°C (2nd wash).

11.   Again discard the upper liquid. Pellet to be processed for DNA extraction should be observed by now at the bottom of the conical. Log-in those that have small amount of pellet.

12.   Add 5 ml TES into each conical and also add 100λ Proteinase K (l0 mg/ml) $\rightarrow$ vortex $\rightarrow$ immerse in a waterbath at 60°C overnight.

DAY 2:

13.   Label a new set of conicals again.

14.   Remove tubes from waterbath and add 5 ml of working Phenol solution.

15.   Vortex these conicals and centrifuge at 3000 rpm @ 10 min.; 4°C.

16.   Harvest the upper phase into a new set of conicals. Add 500λ 3m NaOAc and 10 ml ETOH into each conical. Vortex. Sometimes insoluble DNA can be seen at this stage.

17.   Precipitate the DNA at -20°C overnight or longer.

DAY 3: (final stage)

18.   Centrifuge conicals at 3000 rpm @ 10 min.; 4°C.

19.   Discard supernatant $\rightarrow$ DNA pellet at bottom (difficult to observe).

20.   Invert these conicals to dry the tubes for at least 1 hour. It does not have to be totally dry.

21.   While the tubes are drying, label 3x1.5 ml Eppendorf screw-capped vial per SHS patient with SHS labels. These triplicate vials will be used to store the final DNA.

22.   Add 300 λ TE to each dry conical in order to resuspend the DNA pellet.

23.   Add 20 λ 3M NaOAc to each Eppendorf vials. Add 100λ of resuspended DNA pellet into each vial.

24.   Add 250 λETOH into each vial. Insoluble DNA must be visible by now.

25.   Cap these Eppendorf and separate the triplicates into 3 storage boxes.

26.   Store these vials at -70ºC.

27.   Shipping list with storage box # goes to office for data entry.

QUALITY CONTROL: (for every 10 tubes)

Pipet 300λ of DNA suspended in TE into 498λ of TE. Measure A_{260}. Record the value in the log book.

For every 25 tubes assay DNA by agarose gel electrophoresis.

AGAROSE GEL ELECTROPHORESIS FOR THE EVALUATION OF DNA

The following protocol describes a horizontal electrophoresis of DNA on a low melting Agarose.

Stock Solutions

1.   70% ethanol and 100% ethanol

2.   Proteinase K (10 mg/ml)

3.   Phenol saturated with TES

4.   Chloroform Isoamyl Alcohol (24:1)

5.   3M Sodium Acetate pH 5.21

6.   Glycerol

7.   10% Bromophenol Blue (tracking dye)

8.   10% Xylene cyanol FF (tracing dye)

9.   Tris Base

10. Boric acid

11. EDTA (mw: 372.2)

12. TE (Resuspending buffer)

13. Stock Buffers:

    I. <u>10X TBE</u> (Tris-Borate - EDTA Buffer)

        Prep: Tris-Base   432g
                         Boric Acid   220g
                         0.5M EDTA pH 7.98   160ml
                         $\rightarrow$ Q.S. to 4L with DI-H_2O

    II. Gel Loading Buffer:

        Prep: (6 Fold; Store at 4^{0}C)
              Glycerol   15 ml (Final concentration: 30%)
              10X TBE   5 ml
              Bromophenol Blue 0.25ml of a 10.00% Bromophenol Blue
              Xylene Cyanol FH 0.25ml of a 10.00% Xylene Cyanol FF
              (Final concentration of each dye is 0.25%)
              $\rightarrow$ Q.S. to 50 ml with DI-$H_2$0

    III. Electrophoresis Running Buffer:

        1X TBE + Ethidium Bromide (final concentration is 0.5λ/ml)

14. 0.7% Agarose Gel in 500 ml 1X TBE $\rightarrow$ microwave 20 minutes low temperature. This stock solution can be stored at RT and is reusable over and over again, provided there is no bacterial growth. Pour 100 ml of this 0.7% Agarose into a 100 ml storage bottle and use this as a working stock. Ethidium Bromide (Mutagen and Carcinogen! Be Careful. Wear Gloves and Lab Coat!)

15. HIND III - digest λDNA (from VWR/IBI) $\rightarrow$ molecular weight DNA marker.

    <u>Preparation of DNA marker:</u>

    Example: The Hind-III marker (500λ) comes in with a concentration of 207λ/ml.

    Procedure:

a. Centrifuge the whole 500λ Hind-III marker, 1100 rpm for 5 minutes

b. Mix the Hind-III marker by re-pipetting back and forth

c. Pipet 145λ of Hind-III and mix it with 355λ of TE and 100λ of **Gel** Loading Buffer

d. Final concentration is 50λ/ml

PROCEDURE FOR AGAROSE GEL ELECTROPHORESIS

DAY 1:   Preparation of Samples to be Loaded on Agarose Gel

1. Thaw frozen vials containing DNA tuft. Transfer the DNA tuft into pre-labelled Eppendorf vials. For invisible DNA, centrifuge the vials (11000 rpm for 5 minutes) and discard the supernatant. The DNA pellet will be at the bottom of the vials.

2. To remove inorganic salt and wash the DNA tuft, add 500λ of 70% Ethanol. Centrifuge 11000 rpm for 5 minutes → discard supernatant, invert the vials and air dry for 20 minutes.

3. Add 400 of TES to each vials and 50λ of Proteinase K (10 mg/ml) → vortex → centrifuge 11000 for 1 minute.

4. Resuspend the DNA by repeat pipetting and incubate the vials at 55^0C for 1-hour.

5. After 1-hour, add 400λ of Phenol saturated with TES → vortex → Centrifuge 11000 rpm for 2 minutes.

6. Transfer the upper phase only to a new set of Eppendorf vials. Do not contaminate this DNA containing upper phase with proteins from the intermediate phase.

7. Add 400λ $CHCl_3$ Isoamyl alcohol (24:1) into the vials → vortex → centrifuge for 2 minutes.

8. Again, transfer the upper phase into a new set of Eppendorf vials. Do not transfer the lower phase of $CHCl_3$.

9. Add 40λ 3M NaOAc and 880λ 100% Ethanol into each vial.

10. Store the vials at -70^0C.

11. Thaw the frozen vials (from #10) for 10 minutes centrifuge 11000 rpm for 5 minutes.

12. Wash the DNA by adding 500λ of 70% Ethanol → centrifuge 11000 rpm for 5 minutes → DNA pellet will be visible at the bottom of the vials. If DNA pellet is invisible (for small concentration of DNA) → re-centrifuge for another 5 minutes and proceed to the next step.

13. Discard supernatant and invert the vials. Air dry the vials for 20 minutes.

14. Resuspend the DNA pellet in 100λ of sterile water.

15. To determine the concentration of DNA, pipet 2λ of suspended DNA (from 14) into 498λ of sterile H_2O. Measure A_{260}. Calculation of DNA concentration:

$$(\gamma/\lambda) = \frac{OD_{260} \times 250}{20}$$

DAY 2: Preparation of Agarose Gel Beds for Horizontal Electrophoresis

1. Have the Bio-Rad mini-gel electrophoresis apparatus set clean and ready.

2. Tape the plastic template all around with autoclave tape and fold the other half of the tape underneath the plate to form a mold into which the 0.7% Agarose Gel can be decanted into.

3. Insert the serrated plastic comb into its corresponding grooves (located close to the cathode). Normally, 14 sample wells are formed with the plastic comb.

4. Proceed to pour the melted 0.7% Agarose Gel (microwave the 100 ml stock 3 minutes at high temperature) into the above plastic mold.

5. Once the Agarose has solidified, proceed to remove tape gently as to not tear the agarose off its template. Remove the serrated plastic comb.

6. Insert the plastic tray into the gel cassette.

7. Pour Running Buffer (with Ethidium Bromide in it) into the gel cassette so as to immerse the solidified Agarose.

8.   Form an array of 2λ Gel Loading Buffer on a clean surface of parafilm (use the inside section). Be sure that the amount of 2λ Gel Loading Buffer dispensed on the parafilm corresponds to the amount of samples prepared.

9.   Pipet 1λ of the prepared DNA samples (from #14 Day 1 above) and mix it with the 2λ loading buffer (from #8 above) on the parafilm surface and load the samples into its sample wells. Do this slowly and steadily to prevent puncturing of the gel bed. Also, try to avoid releasing the samples outside of the sample wells.

10.   The extreme left sample well is usually allotted for the Hind-III DNA markers. Add 4λ of the Hind-III marker straight into its slot (without mixing with loading buffer).

11.   Be sure that the electrophoretic apparatus is connected properly to the power supply. Turn on the power and the running voltage is usually 50V for 20 minutes.

Photography of DNA

Ethidium Bromide is an intercalating agent between stacked bases and fluoresces of under illumination.

Procedure:

Once the electrophoresis is over, remove the whole plastic tray (6 above) and put it on top of the transilluminator (Model UVT 750-M/IBI).

Reminder: Put on anti UV goggles while viewing the illuminated dry gel. THIS IS A MUST.

Intact DNA will show only a single band. Proceed to take a Polaroid (#667) picture of the gel. Label the picture and put it in the record book.

NB: For QC Purposes:

1. A_{260} measurement is performed on every 10th sample.

2.   Agarose gel electrophoresis is done on every 25th sample.

1.5.14 Lipoprotein(a) Assay (Phase I samples only)

INTRODUCTION:

Lipoprotein(a) is a cholesterol-rich plasma lipoprotein with pre-beta electrophoretic mobility. Lp(a) contains, in addition to Apolipoprotein B, the disulfate-liked Apolipoprotein (a).

Apo(a) shares extensive structural homology with plasminogen, including multiple repeating domains similar to "kringle" four, a single region similar to "kringle" five and an inactive protease segment. Recent clinical studies suggest that Lp(a) may be an independent risk factor for coronary heart disease. Even though Lp(a) generally makes up less than 15% of the total plasma cholesterol, a high concentration of Lp(a) in plasma is associated with a twofold to fourfold increased risk of premature coronary heart disease.

METHODOLOGY AND PROCEDURE SUMMARY:

The Macra™ Lp(a) enzyme immunoassay kit is obtained from Terumo Medical Corporation. Four controls are run on each plate:

TL1 and TL2 from Terumo
7517 from International Enzymes
YCLC (in house)

Plates are washed using an Automated Microplate Washer (Bio-Tek EL 402) and read on a Microplate OD Reader (Bio-Tek EL 309).

Samples, YCLC and 7517 controls are diluted in two steps: 1:201 and 1:26 with MLK diluent, standards and MLK controls (TL1 and TL2) are diluted 26 times with MLK diluent. 100 μl of diluted standards, controls, and samples are pipetted into the pre-coated Macra™ Lp(a) plates. Plates are incubated at 27°C for 2 hours. Plates are washed 5 cycles with MLK washing buffer using automated microplate washer. 100 microliter of anti-Lp(a)-HRP conjugate are added to the wells in each plate. Plates are incubated at 27°C for 90 minutes. Plates are washed 5 cycles with MLK washing buffer. 100 μl of substrate (made from colorimetric reagent and OPD tablets prepared 20 minutes prior to use) are dispensed into each well. Plates are incubated at 27°C until the yellow standard 80 is dark enough to give a reading of 1.5 on DO_{495nm} (60-90 min.) 50 μl of stop reagent is dispensed into plates and Plates are read at 495 nm on Microplate OD Reader. The results are calibrated from the linear regression of the standards. Results outside the range of the curve are to be repeated.

The incubation temperature and humidity should be tightly controlled. Antigen and antibody binding is sensitive to temperature. Lower temperature will delay equilibrium, while higher temperature will change linear proportional range of assay. On the other hand, a humid chamber can stabilize the antigen-antibody binding and reduce evaporation during incubation.

Assay precision

The average coefficient of variation of intra-assay for 4 fresh and 4 frozen samples was 8.39%. The average coefficient of variation of inter-assay for 4 frozen samples was 9.39%.
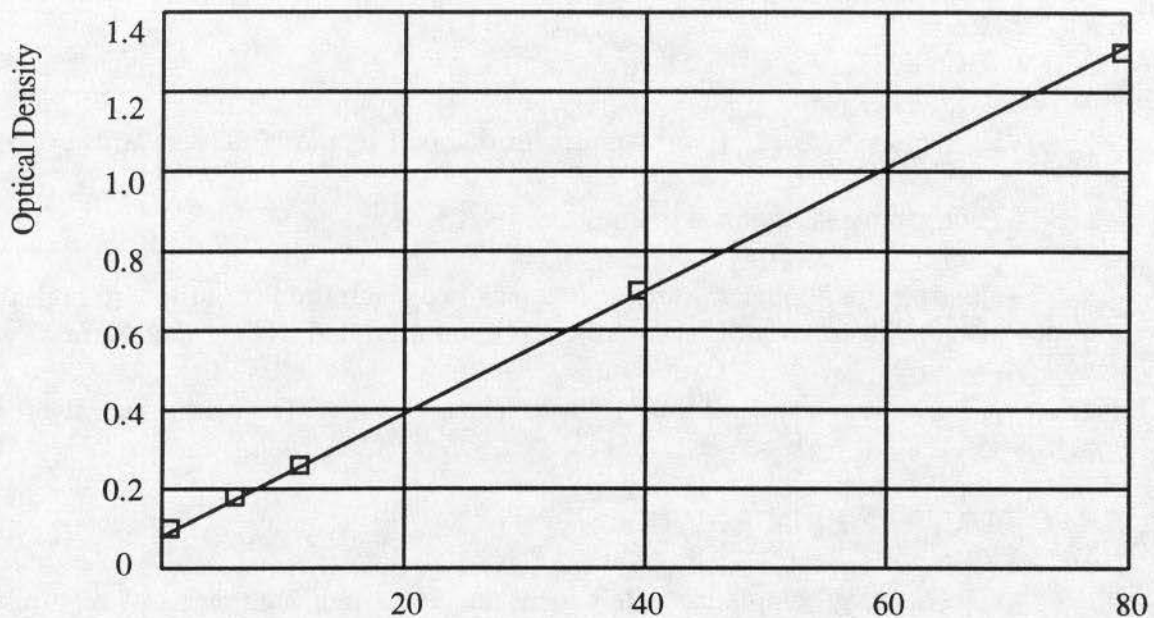
Figure 4. A Typical Calibration Curve for the Modified ELISA Lp(a) Assay

Assay Accuracy:

A known amount of purified Lp(a) fraction was added into different plasma samples with known Lp(a) concentration. The recovery for each combination was calculated from expected and observed values. The average recovery was 102.99%, while recovery range was 91.94% to 112.51%.

Normal Range:

< 20 mg/dl

TESTING SAMPLES:

DILUTIONS:

1. YCLC and 7517 controls are diluted in two steps: 1:201 and 1:26.

2. TL1 and TL2 controls are diluted 1:26.

2. All samples except SHS are diluted in two steps: 1:201 and 1:26.

3. All SHS samples are diluted 1:1000.

4.   All standards (0,5,10,20,80) are diluted 1:26.

PLATE-SET UP:

1.   Each plate holds 22 patient samples in addition to controls and standards.

2.   Each sample is aliquoted in triplicate, 100 μL each.

3.   Controls are aliquoted in reverse order of concentration (from low to high.) The same holds true for the standards, (i.e. aliquot std 0, 5, 10, 20, then 80 last.)

4.   A fresh set of standards must be used each set up day. The controls are stable for 1 week.

GENERAL PLATING PROCEDURES:

1.   Fill out assay sample list with patient numbers. Indicate repeats by recording an "R" prior to the patient number. Indicate any dilution variations from the norm.

2.   Label all test tubes needed for both 1st and 2nd dilutions. Use a different colored ink for each set.

3.   Dilute all samples, controls, and standards.

4.   Pipet 100 μl of each control into appropriate wells on all plates in ascending concentration order.

5.   Pipet 100 μl of each standard into appropriate wells on all plates in ascending concentration order.

6.   Pipet 100 μl of each sample into appropriate wells.

7.   Incubate plates for 2.0 hours.

8.   Prime the pump on the plate washer then wash each plate 5 cycles with washing buffer. Tap out excess wash solution from the plates.

9.   Add 100 μl of the conjugate solution into each well.

10.   Incubate the plates for 90 minutes.

11. Twenty minutes prior to the end of the incubation period prepare color developing solution by dissolving OD tablets into colorimeter reagent. (1 table per 5 ml of solution; prepare 20 ml to ensure adequate amount).

12. At the end of the incubation, wash each plate 5 cycles with washing buffer. Tap any excess wash solution from the plates.

13. Add 100 µl of color developing solution to each well.

14. Incubate the plates at 27°C. After 1.0 hour check the each plate by reading on Mode 7. If the STD 80 value reads at least .35 the plate is ready. If the value is less that .35 continue to incubate the plate. Recheck periodically until a reading of at least .35 is obtained.

15. Add 50 µl of stop reagent to each well.

16. Read plates on Mode 5 (wavelength = 490 nm). The resulting readings are to be copied onto a floppy disk for analysis.

17. Analyze and the data using Maciel RIA-PC Version 5.0.

SAMPLE REPEAT CRITERIA:

Concentration > 75 mg/dl:	repeat using a 201-26 two step dilution
Concentration > 135 mg/dl:	repeat using a 401-26 two step dilution
CV% > 15%:	repeat using 1:1000 dilution.

QC ACCEPTANCE CRITERIA

Mark all control values on Levey-Jennings plots. Follow the standard Westgard Rules listed below for flagging or rejecting plates:

The ranges for controls are as follows:

Control	MRI-AVG	±2SD
TL1	17.30	15.19 - 19.59
TL2	35.90	30.10 - 41.30
YCLC	8.55	6.11 - 11.10
7517	59.90	41.90 - 77.90

STANDARD WESTGARD RULES

Rule	Variation observed	Action Taken
1_{2s}	One control sample exceeds the mean by $\pm$ 2SD	Warning only. Chart and flag.
1_{4s}	On control sample exceeds the mean by $\pm$ 4SD	Reject plate. Possible random error.
2_{2s}	Two control samples vary from the mean by $\pm$ 2SD, both in the same direction	Reject plate. Possible systematic error.
R_{4s}	One control sample exceeds the mean by + 2SD and another is below the mean by 2SD.	Reject plate. Possible random error.
4_{1s}	Four consecutive controls all falling on the same side of the mean.	Warning only. Suspect systematic error.
10_x	10 consecutive controls all falling on the same side of the mean.	Reject plate. Suspect systematic error

1.5.15 LDL Subclasses (Phase I samples only)

Introduction: Low Density Lipoproteins (LDL) are the major cholesterol-carrying lipoproteins in plasma. LDL includes a spectrum of particles differing in size, density, and composition. Characterization of LDL subclasses is important to the interpretation of decreased LDL particle size associated with premature coronary artery disease and to basic studies of metabolic origins and interconversion of LDL.

Principle: From gel chromatography experiments Fawcett and Morris[1] found that the pore size (S_{poor}) of polyacrylamide gel varied inversely with and approximately linear to the total monomer concentration (T_{gel}). That is $T_{gel} \times S_{gel} = C_{constant}$. Gradient gell electrophoresis entails the migration of charged particles through a matrix comprised of an increasing concentration of polyacrylamide gel. The migration rate of particles with decreasing particles size ($S_{particle}$) progressively approaches zero as the gel concentration increases. With spherical particles, when a pore size is reached which is just smaller that the particle, the migration of that particle is stopped. The pore size at this point (S_{pore}) is approximately equal to the particle size ($S_{particle}$). Using this comparison, the previous equation can be altered to read $T_{gel} \times S_{particle} = C_{constant}$. If the particle size and gel concentration are known the constant for each gel can be calculated. Using calibrators with known particle size the concentration of different gel positions can be calculated. The particle size of an unknown sample can then be calculated:

$$S_{(particle)} = C_{constant}/T_{gel}$$

1.6   Quality Assurance Program

1.6.1 Collecting QA Samples

In addition to the use of quality controls with each run, there is a need to assure that all the steps from blood drawing to sample receiving and laboratory measurements are correct. Therefore, replication of unknown samples will be necessary.

At the discretion of the individual PI, one participant the first clinic day every week will serve as the replicate control - the recommended scheme is to select the first participant who has a GTT for blood drawing that clinic day. If there is more than one clinic site, they should be rotated so that QA samples are sent from all clinic sites with the same frequency. A check list for blood drawing for quality control sample is available (Appendix 2). A total of two urine tubes and five extra blood tubes will have to be collected. The blood tubes are as follows:

<div style="padding-left: 2em;">

Fasting: 2 [10-ml] Lavender top tube
                1 [5-ml] Gray top tube
                1 [4.5-ml] Blue top tube
                1 [9.5-ml] Red top (SST) tube

</div>

These are indicated on the Check List.

The numbering system for these quality control samples is similar to the Study ID and consists of 6 digits with the first digit corresponding to the center (1-SD, 2-OK, 3-AZ), the second digit will be a "3" to indicate that the sample is a QA and a 4-digit sequence number. A complete set of tubes should be expected for QA samples. The Coordinating Center should receive at monthly intervals the list matching Study ID to QA for analysis (Appendix 2). This list should not be made available to the Core Laboratory until results for the samples and QA duplicates are reported. (Appendix 2).

1.6.2 Processing QA samples

The flow diagram in figure 11.6 illustrates the blood and urine processing procedure for QA samples.

1.   Label vials and tubes for each patient as follows (this should be done before beginning the blood processing). Attach the labels according to the labelling diagram in figure 11.6.1. All tubes are to be labelled with the appropriate QA ID label before processing is begun:

Num/Pat	Container	Label	Storage
1	14-ml plasma tube	LIPIDS	refrigerated (4°C)
2	2-ml Cryovials without inserts	HbA1c	frozen(-70°C)
3	2-ml Cryovial with blue cap insert	COAG	frozen (-70°C)
2	2-ml Cryovials with white cap inserts	INSULIN	frozen (-70°C)
2	14-ml Urine Tube	URINE	frozen (-70°C)
2	2-ml Cryovial with yellow cap inserts	0HR GLUC	frozen (-70°C)

Note: There are no QA samples for Storage, Buffy Coat, or 2hr Glucose

2. Arrange the vials and tubes in the above order on ice.

3. Spin all blood tubes at 4°C for 10 minutes, at 3000 RPM (1000 x g).

4. Remove tubes from centrifuge and place them in a rack on ice.

5. [10-ML] LAVENDER TOP TUBES (total of 2)

   A. Remove stoppers from both [10-ml] lavender top tubes.

   B. With a fresh, disposable transfer pipet, place approximately 6-7 ml into the 14 ml plasma tube.

   C. Do not freeze the [14-ml] plasma tube, place in refrigerator for weekly shipment. Make sure that the cap has been securely closed. Do not freeze this tube.

   D. Transfer at least 1.5 ml of red cells from the last tube into each of the 2 remaining [2-ml] Cryovials without cap inserts labelled "HbA1c." Cap and freeze these tubes as quickly as possible at -70°C.

   E. Replace the stopper in the other vacutainer tube. Label this tube with the QA ID label for "Red Cells" and place tube in refrigerator for weekly shipment to Memorial Blood Center of Minneapolis (see shipping instructions in section 11.4.) Make sure that the stopper has been securely replaced. Do not freeze this tube.

   F. Discard the used pipets and the remaining tube with the red cells.

6. BLUE TOP TUBE

   A. Remove the stopper from the tube.

   B. With a fresh transfer pipet, divide all of the plasma between the three [2-ml] Cryovials labelled "Coag."

   C. Using caps with a blue insert, securely cap the vials.

   D. Freeze the vials as quickly as possible at -70°C. Once frozen, the samples are to be kept at -70°C until shipment.

   E. Discard the pipet and tube with the remaining red cells.

7. RED TOP (SST) TUBE

   A. Remove the stopper from the tube.

   B. With a fresh transfer pipet, divide the plasma between the two [2-ml] Cryovials labelled "Insulin."

   C. Using a caps with white inserts, securely cap the vials.

   D. Freeze the vials as quickly as possible at -70°C. Once frozen, the samples are to be kept at -70°C until shipment.

   E. Discard the pipet and tube with the remaining red cells.

8. URINE SAMPLE (Use aliquots from the same sample collected for routine testing.)

   A. Using a pipet, transfer approximately 8 ml of the patient's urine sample into each of 2 the tubes labeled "URINE". Discard the remaining sample.

   B. Securely cap the tubes and freeze at -70°C.

   C. Once frozen, the samples are to be kept at -70°C until shipment.

9. GRAY TOP TUBE

   A. Remove the stopper from the fasting [5-ml] gray top tube.

B. With a fresh transfer pipet, divide the plasma between the two [2-ml] Cryovials labelled "0Hr Gluc."

C. Using caps with <u>yellow</u> inserts, securely cap the vials.

D. Discard the pipet and tube with the remaining red cells.

E. Freeze the vials as quickly as possible at -70°C. Once frozen, the samples are to be kept at -70°C until shipment.

F. Discard the pipets and tubes with the remaining red cells.

10. Three zip-lock bags will be needed for each participant.

Bag A will be used for the refrigerated shipment to Penn Medical Laboratory (Medlantic Research Institute) and should contain the following properly labelled tube:

* 1 [14-ml] plasma tube ("Lipids")

Bag B will be used for the shipment to Memorial Blood Center of Minneapolis and should contain the following properly labelled tube:

* 1 [10-ml] LTT Vacutainer tube ("Red Cells")

Bag C will be used for frozen shipment to Penn Medical Laboratory (Medlantic Research Institute) and should contain the following properly labelled tubes:

* 2 [2-ml] Cryovials ("HbA1c")
* 3 [2-ml] Cryovial with blue insert ("Coag")
* 2 [2-ml] Cryovials with white inserts ("Insulin")
* 2 [14-m] Urine samples ("Urine")
* 2 [2-ml] Cryovials with yellow inserts ("0Hr Gluc")

1.6.3 Shipping QA Samples

QA samples are to be included with the regular shipments. See section 11.4 for shipping instructions.

FIGURE 5. Processing Blood Samples and Urine Sample

FASTING

Two [10-ml]
Lavender top tubes

Centrifuge
(1500 RPM, 10 min 4ºC)

Plasma ——————— Red Cells

1 x [14-ml] tube
(apx 7 ml)
DO NOT FREEZE
ship cold (blue ice)
("Lipids" label)

Aliquot 2 x [2-ml]
Cryovials for HbA1c
(store/ship frozen)
("HbAlc" label)

Red Cells for typing
("Red Cells" label)
DO NOT FREEZE

One [5-ml]
Gray top tube

Centrifuge
(1500 RPM, 10 min 4ºC)

Plasma (G0)
2 x [2-ml] Cryovials
with yellow cap inserts
("0Hr Gluc" label)
(store/ship frozen)

One [9.5-ml] SST
Red (tiger) tube

Centrifuge
(1500 RPM, 10 min 4ºC)

Serum
2 x [2-ml] Cryovials
with White cap inserts
("Insulin" labels)
(store/ship frozen)

One [4.5-ml]
Blue top tube

Centrifuge
(1500 RPM, 10 min 4ºC)

Plasma
3 x [2-ml] Cryovials
with blue cap insert
("Coag" labels)
(store/ship frozen)

One Urine
sample

DO NOT
CENTRIFUGE

apx 8 ml
each of
two Urine
transfer
vials
("Urine" label)
(store and
ship frozen)

ATTACHING BAR CODED LABELS
TO SAMPLE VIALS

Small Vials (Cryovials)

Attach label with lines in bar
code in horizontal orientation.
(see diagram)

Labelled vial

Large Vials (SC Tubes)

Attach label with lines in bar
code in horizontal orientation.

Allign label with top of vial.
(see diagram)

Labelled vial

FIGURE 6.   ATTACHING BAR CODED LABEL TO SAMPLE VIALS

1.7   Sample Handling and Storage at the Core Laboratory

Note: Gloves and glasses must be worn when handling samples. An appropriate barrier must be used at any time there is a risk of aerosols (such as when opening sample tubes.)

1.7.1 Procedure for Handling Refrigerated Sample Shipment

The following procedure will be followed for shipments sent on **Blue-Ice** from the various clinics.

1.   Shipping Box is received.

2.   Check off the enclosed Shipping List making sure that ID's on the tubes match with the ID's on the Shipping List.

There should be a separate zip-lock bag for each subject containing the following tube:

        *   1 [14-ml] plasma tube ("Lipids")

In addition a set of extra self-sticking pre-printed Id#'s corresponding to the samples sent should also be included in the box.

3.   Set up the plasma sample for lipid measurements and ultracentrifugation.

1.7.2 Procedure for Sorting and Logging Frozen Specimens

1.   Frozen samples will arrive in a container with dry ice. Observe the condition of the samples. If any evidence of thawing is present, it should be reported to the Center of Origin and noted on the shipping list.

2.   Have on hand sufficient insulated container(s) to hold Eleven (11) cardboard freezer boxes (labelled "Storage," "Buffy Ct," "Insulin," "Coag," "Coag Store," "Glucose," "HbA1c," "Special," "Special Store," "Urine," and "Urine Store.")

3.   Transfer dry ice to the insulated container(s) sufficient to keep the samples frozen during processing.

4.   Have on hand the accompanying shipping list from the sender and storage box logs.

5. This process will require two (2) people, one to sort samples, and the other to record SHS numbers and appropriate information on the logging slips.

6. Open the shipping container. Samples from each individual patient are stored in separate plastic bags. Each bag should contain the following Cryovials:

>   5 x 2 ml with no cap inserts ("Storage")
>   1 x 14 ml ("Buffy Ct")
>   2 x 2 ml ("HbA1c")
>   3 x 2 ml with blue cap inserts ("Coag")
>   2 x 2 ml with white cap inserts ("Insulin")
>   2 x 2 ml with yellow cap inserts ("0Hr Gluc")
>   2 x 2 ml with red cap inserts ("2Hr Gluc")*
>   3 x 2 ml with green cap inserts ("Special"
>   2 urine vials ("Urine")

    * Participants exempted from the GTT will not have 2Hr Gluc samples

7. Remove one plastic bag at a time from the shipping container and count the number of tubes including urine tubes. These should be checked off by patient number on the shipping list by the second person. After being checked off, the sorting process will begin. Any discrepancies should be recorded on the shipping list.

8. **Sorting Process**: Remove the vials one by one from each bag. Check the tubes to ensure the labels are properly and securely attached. Sort the tubes in the boxes as follows (see diagram):

Samples for testing at PML

One set each of 0Hr Gluc (yellow caps) and 2Hr Gluc (red caps) into the "Glucose" box

>   One 2 ml white cap tube in the "Insulin" box
>   The Buffy Coat tube (14 ml) in the "Buffy Ct" box

Samples to be shipped to other labs

>   One Urine vial in the "Urine" box
>   One HbA1c vial in the "HbA1c" box
>   Two (2) blue cap tubes in the "Coag" box
>   Two (2) green cap tubes in the "Special" box

Samples for storage

The plasma samples (no inserts) in the "Storage" box

The second set of glucose tubes in the "Storage" box (next to the plasma samples for the same patient)

The second white cap tube in the "Storage" box (next to the other samples for the same patient)

One HbA1c vial in the "Storage" box (next to the other samples for the same patient)

One Urine vial in the "Urine Store" box

One blue cap in the "Coag Store" box

One green cap tube in the "Special Store" box

9. Repeat this process for each of the bags and be sure to record any missing vials on the accompanying shipping list.

10. Sort the QC samples in the same manner as the regular patient samples.

11. When the sorting is complete, store the cardboard boxes on their appropriate shelves in a -70°C freezer.

12. Coag, HbA1c and Urine boxes should be placed on the bottom shelf while waiting to be shipped out for assays.

13. Record sample numbers on box log sheet.

14. Fax a copy of the shipping log sheet to the Center PI, with any discrepancies clearly recorded.

15. Be sure to send back the original shipping container to the appropriate center by UPS (ground).

16. Shipping logs go to office for entry onto data set.

17. At this point, sample processing is completed.

1.7.3. Procedure for Shipping Frozen Samples for Analysis

1. Shipping out of samples will be done only on Tuesdays.

2. When 2 to 3 boxes for each type of analyte are available, then those boxes can be shipped for analyses.

3.   Place the boxes of urine, etc., into insulated containers with dry ice. Urine and HbA1c samples are to be packed together in one box. Coag and Special samples are to be packed together in a second box.

4.   Inside the box lid of each shipping container, place a list of samples to be analyzed.

5.   Seal the container tightly with strong sealing tape.

6.   Fill out the appropriate Federal Express form, insert it inside the mailing pouch and attach it to the top of the container.

7.   Place a separate address label (with phone numbers) on the side of the container.

8.   Complete and attach an "ORM-A" (dry ice) label on the outside of the box.

9.   Call Federal Express (301/953-3333) and tell them you have a package pick-up. Fedex will require an account number, weight of boxes to be picked up and zip code of the receiving Center. The destination of receiving Centers are as follows:

   Urine/HbA1c: Ms. Linda Phillips
                 NIH, STRONG HEART STUDY
                 1550 E. Indian School Road
                 Phoenix, AZ 85014
                 602/263-1615

   Coag/Special: Russell Tracy, Ph.D.
                 Department of Pathology
                 University of Vermont
                 55A South Park Drive
                 Colchester, VT 05446
                 802/656-8961

10. Fedex will give you a confirmation number. Record this number on the box.

11. Fedex will come and pick up the boxes before 5 pm the same day.

12. Call the appropriate receiving center and notify them of the impending shipment.

FIGURE 7.   ROCESSING SAMPLES AT CORE LAB

```
┌─────────────────────────────────────────┐
│           Refrigerated Sample            │
│         1 [14-ml] Plasma Tube            │
│                   │                       │
│                   │                       │
│          Set for β-Quantification         │
│              DO NOT FREEZE                │
└─────────────────────────────────────────┘
```
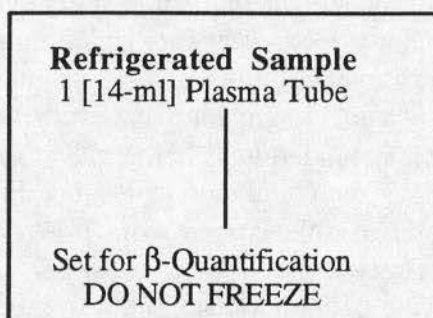
```
┌───────────────────────────────────────────────────────────────────────────┐
│           Frozen Samples (Keep all samples frozen until testing)            │
│                                                                             │
│  5 x [2-ml] Plasma        1 x [14-ml] Buffy Coat      2 x [2-ml] Serum      │
│         │                         │                   (White cap insert)    │
│                                                              │              │
│       Storage                   DNA                  1 sample for Insulin   │
│                                                      1 sample for Storage   │
│                                                                             │
│                                                                             │
│  3 x [2-ml] Plasma        2 x [2-ml] Red cells        2 x [14-ml] Urine     │
│  (Blue cap insert)                │                          │              │
│         │                                                                   │
│    2 Samples                                                                │
│  Repack for shipment      Repack for shipment        Repack for shipment    │
│  (Fibrinogen)             (HbAlc)                                           │
│                                                                             │
│                                                                             │
│  2 x [2-ml] Plasma        2 x [2-ml] Plasma           3 x [2-ml] Plasma     │
│  (Yellow cap insert)      (Red cap insert)            (Green cap insert)    │
│         │                         │                          │              │
│  One sample for glucose   One sample for glucose     2 samples Repack for   │
│  One sample for storage   One sample for storage     shipment (Special)     │
│  (G-0)                    (G-2)                      1 sample for storage   │
└───────────────────────────────────────────────────────────────────────────┘
```

1.8   Infection Control Policy

The Strong Heart Study recognizes that some study subjects might possibly be infected with Hepatitis virus, HIV or other infectious agents which might potentially be transmittable to either clinical or laboratory personnel handling blood specimens. All blood samples should be handled with great caution. It is safest to presume that every sample is potentially harmful. It is not feasible in a field study to pretest individuals before the examination, nor should they properly be excluded if a random sample of the population is desired. Furthermore, no laboratory test can reliably determine if any blood sample is free from infectious agents. Since contact with individuals with infectious diseases can pose risk to study personnel, a policy has been developed for both clinical personnel during patient contact and also laboratory personnel handling blood specimens. These policies are attached to appendix 16 in Volume II.

APPENDIX 1

THE STRONG HEART STUDY II

Routine Blood and Urine Sample Collection Checklist

Participant's Name

Today's Date (mm/dd/yy) ☐☐ / ☐☐ / ☐☐   ID Number

Fasting Samples *

☐ 3 [10-ml] Lavender   Lipids/Buffy Ct/Red Cells/HbA1c

☐ 1 [5-ml] Gray   Glucose/Creatinine

☐ 1 [4.5-ml] Blue   Fibrinogen/PAI-I/CRP

☐ 1 Special tube   Special Coag Studies (TBD)

☐ 1 [9.5-ml] Red (SST)   Insulin

Urine

☐ 2 [14-ml] tube   Microalbumin./Creatinine

Two Hour Sample

☐ 1 [5-ml] Gray*   Glucose

---

* If the participant is exempt from the GTT procedure, the 2 hour sample will not be drawn.

APPENDIX 2
THE STRONG HEART STUDY II

Quality Control Blood Collection Checklist

Participant's Name _____

Today's Date (mm/dd/yy) ☐☐/☐☐/☐☐

ID Number [_____]   Quality Control ID Number [_____]

Fasting Samples

☐	3 [10-ml] Lavender	Lipids/Buffy Ct/Red Cells/HbA1c
☐	1 [5-ml] Gray	Glucose/Creatinine
☐	1 [4.5-ml] Blue	Fibrinogen/PAI-I/CRP
☐	1 Special tube	Special Coag Studies (TBD)
☐	1 [9.5-ml] Red (SST)	Insulin

Quality Control Samples

☐	2 [10-ml] Lavender	QC Lipids/Red Cells/HbA1c
☐	1 [5-ml] Gray	QC Glucose/Creatinine
☐	1 [4.5-ml] Blue	QC Fibrinogen/PAI-I/CRP
☐	1 [9.5-ml] Red (SST)	QC Insulin

Urine (One sample divided as follows)

☐	2 [14-ml] tube	Microalbumin./Creatinine
☐	2 [14-ml] tube	QC Microalbumin./Creatinine

Two Hour Samples

☐	1 [5-ml] Gray*	Glucose

---

* The first participant of one clinic day every week will be selected for QC. The above will have to be obtained from that participant and sent with the regular shipments.

APPENDIX 3
STRONG HEART STUDY II

Flow of Samples for Two Participants Using the Workstation

Gray top tube (Fasting)

Plasma

Discard cells

α - Glucose
Creatinin

α - Glucose
Creathon

ICE

TRAY

1 2 3 4 5 6

1 6 Go — — — A
2 7 Go — — — B
3 8 — — — — C
4 9 G2 — — — D
5 10 G2 — — — E

Δ14 [2-ml]
Cryovials
-70°C

ΔUrine
(-70°C)

14-ml
Plasma tube

*Plasma
4°C

Plasma

Discard cells

Gray-top tube
(2-hour)

2-hr Glucose

*[5-ml] lavender
top tube
(2-hour) 4°C

3 [10-ml] Lavender-
top tubes (fasting)

*Recap tightly
4°C

* Ship cold, blue ice.
Δ Ship frozen, dry ice
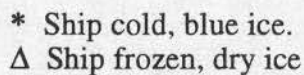
THE STRONG HEART STUDY II
CARDIOVASCULAR DISEASE IN AMERICAN INDIANS

Cold Sample Shipment Slip

Center ID: _____   Contact Person: _____

Today's Date: _____   Technician ID: _____

Comments:

				LABORATORY USE ONLY				
				Rec Dt: __/__/__ Rec by: _____				
NO	ID Number	Drawn	14 ml PL	LABSEQ	SAMPLE COND			BOX
01			[ ]		NILH	A N	L F	
02			[ ]		NILH	A N	L F	
03			[ ]		NILH	A N	L F	
04			[ ]		NILH	A N	L F	
05			[ ]		NILH	A N	L F	
06			[ ]		NILH	A N	L F	
07			[ ]		NILH	A N	L F	
08			[ ]		NILH	A N	L F	
09			[ ]		NILH	A N	L F	

THE STRONG HEART STUDY II
CARDIOVASCULAR DISEASE IN AMERICAN INDIANS

Sample Shipment Slip - Paternity Lab - Memorial Blood Center

Center ID:_____   Contact Person:_____

Today's Date:_____   Technician ID:_____

LABORATORY USE ONLY		
Rec Dt: ___/___/___		
Rec by: _____		

NO	ID Number	Drawn	Red Cells	EDTA RECD	CONDITION	COMMENTS
01			[ ] [ ]	EDTA RECD	CONDITION [ ] OK	COMMENTS
02			[ ] [ ]	EDTA RECD	CONDITION [ ] OK	COMMENTS
03			[ ] [ ]	EDTA RECD	CONDITION [ ] OK	COMMENTS
04			[ ] [ ]	EDTA RECD	CONDITION [ ] OK	COMMENTS
05			[ ] [ ]	EDTA RECD	CONDITION [ ] OK	COMMENTS
06			[ ] [ ]	EDTA RECD	CONDITION [ ] OK	COMMENTS
07			[ ] [ ]	EDTA RECD	CONDITION [ ] OK	COMMENTS
08			[ ] [ ]	EDTA RECD	CONDITION [ ] OK	COMMENTS
09			[ ] [ ]	EDTA RECD	CONDITION [ ] OK	COMMENTS

APPENDIX 5

THE STRONG HEART STUDY II
CARDIOVASCULAR DISEASES IN AMERICAN INDIANS

Frozen Sample Shipment Slip

Center ID: _____   Contact Person: _____

Today's Date: ___ / ___ / ___   Technician ID: _____

					LABORATORY USE ONLY		
					Rec Dt: ___ / ___ / ___ Rec by: _____		

No	ID Number	I	D	Drawn	STOR	BUFF	HbAlc	Coag	Insu	Urin	Gluc	LABSEQ	SAMPLE COND			BOX
													NILH	AN	LF	
01							2 Hour Sample						NILH	AN	LF	
													NILH	AN	LF	
02							2 Hour Sample						NILH	AN	LF	
													NILH	AN	LF	
03							2 Hour Sample						NILH	AN	LF	
													NILH	AN	LF	
04							2 Hour Sample						NILH	AN	LF	
													NILH	AN	LF	
05							2 Hour Sample						NILH	AN	LF	
													NILH	AN	LF	
06							2 Hour Sample						NILH	AN	LF	

1. Record the number of tubes shipped in the space indicated.
2. Mark in the appropriate column for patients on Insulin (I) or Dialysis (D).

THE STRONG HEART STUDY -- PHASE II
CARDIAVASCULAR DISEASE IN AMERICAN INDIANS

Urine LOG

Date sent: _____   Technician: _____

Seq	ID	Rec'd	Comments	Seq	ID	Rec'd	Comments
1				21			
2				22			
3				23			
4				24			
5				25			
6				26			
7				27			
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16				36			
17				37			
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19				39			
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APPENDIX 7

THE STRONG HEART STUDY -- PHASE II
CARDIAVASCULAR DISEASE IN AMERICAN INDIANS

Insulin LOG

Date sent: _____   Technician: _____

Seq	ID	Rec'd	Comments	Seq	ID	Rec'd	Comments
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14				34			
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16				36			
17				37			
18				38			
19				39			
20				40			

APPENDIX 8

THE STRONG HEART STUDY -- PHASE II
CARDIAVASCULAR DISEASE IN AMERICAN INDIANS

DNA LOG

Date sent: _____   Technician: _____

Seq	ID	Rec'd	Proc'd	Box#	Seq	ID	Rec'd	Proc'd	Box #
1					21				
2					22				
3					23				
4					24				
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15					35				
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18					38				
19					39				
20					40				

APPENDIX 9

THE STRONG HEART STUDY -- PHASE II
CARDIAVASCULAR DISEASE IN AMERICAN INDIANS

Fibrinogen LOG

Date sent: _____   Technician: _____

Seq	ID	Rec'd	Comments	Seq	ID	Rec'd	Comments
1				21			
2				22			
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STRONG HEART STUDY

ND

SD

AZ

OK

**CARDIOVASCULAR DISEASE IN AMERICAN INDIANS
(PHASE II)**

OPERATIONS MANUAL - VOLUME FOUR

SPECIAL EXAMINATIONS

THE NATIONAL HEART, LUNG AND BLOOD INSTITUTE
OF THE NATIONAL INSTITUTES OF HEALTH

THE STRONG HEART STUDY

Cardiovascular Disease in American Indians
(Phase II)

Operational Manual

Volume Four

Special Examinations

July 1, 1993

For copies, please contact

Strong Heart Study Coordinating Center

Center for Epidemiologic Research

University of Oklahoma Health Sciences Center

P.O. Box 26901
Oklahoma City, OK 73190

MANUAL IV

Special Examinations

1. ECHOCARDIOGRAPHY READING CENTER
MANUAL OF OPERATIONS

1.1 Goals of Study

Echocardiographic measurement of left ventricular (LV) mass, function and blood flow characteristics in clinical and epidemiologic studies has improved the understanding of the prevalence, demographic correlates, and prognostic significance of preclinical and clinically evident heart disease. Echocardiographic LV mass, which is increased by high blood pressure, obesity and other risk factors or life-style variables, has been shown to be an extremely strong predictor of subsequent morbidity and mortality. Genetic influences on LV mass have been suggested by family studies and by differences between African-Americans and whites in cardiac anatomy and hemodynamics. Native Americans constitute a segment of the U.S. population with high prevalences of obesity, diabetes and other risk factors and apparently rising rates of cardiovascular morbidity in which virtually nothing is known of the prevalence or correlates of structural changes of the heart and blood vessels in asymptomatic individuals that may be termed "preclinical cardiac disease." Accordingly, echocardiography will be used in American Indian participants in Exam II of the Strong Heart Study (SHS) in 3 areas (Arizona, Oklahoma and South Dakota) to answer the following questions:

1) What is the distribution of LV size, mass and function among Native Americans, and does it differ from findings in Caucasian populations?

2) What is the association of LV mass and cardiac function to prevalent, clinically recognized heart disease among Native Americans?

3) Is percent body fat and its distribution an independent predictor of LV enlargement independent of the effects of lean body mass or blood pressure?

4) What are the cardiac functional characteristics and prevalence of the cardiomyopathy of diabetes, and can its early features be detected in individuals with impaired glucose tolerance and a low likelihood of atherosclerotic disease?

5) Are circulating insulin levels associated with LV mass, independent of conventional stimuli to cardiac growth?

6) Is alcohol intake associated with LV hypertrophy and at what level of intake does LV dysfunction become evident?

7) What are the prevalence of and factors associated with valvular heart disease among Native Americans?

The populations participating in the SHS present a unique opportunity to answer these questions because of the particular mix of genetic and environmental factors and because of the relatively under-studied status of cardiovascular disease in this important ethnic group.

1.2   Background of the Study

Over the past 4 decades the understanding of cardiovascular disease has been greatly enhanced by epidemiologic studies in which risk factors have been related to prevalent (already recognized) and incident (newly occurring) cardiovascular disease. Despite its fruitfulness, this research strategy is limited by the commonly long latent period before risk factor exposure leads to morbid events, and the inconsistency with which exposure to such strong risk factors as elevated blood pressure or cholesterol levels leads to morbid events even during observation periods as long as 20 years. A series of methodologic developments have led to a conceptual advance, in which "preclinical disease" detectable by noninvasive methods has been shown to be an intermediate step between risk factor exposure and development of morbid events.

Although a number of methods may detect preclinical cardiovascular disease, including ultrasonic visualization of arterial atherosclerosis (1) and measurement of microalbuminuria (2), the measure of preclinical disease that has been most extensively validated and studied in clinical and epidemiological contexts is echocardiographic LV mass (3-5). A variety of echocardiographic methods have been shown to measure human LV mass accurately by necropsy comparison (6-10), and this measurement has been shown to be an extremely strong predictor of cardiovascular morbidity and mortality in clinical and general population samples (3-4, 11-15). The echocardiographic examination to measure LV mass can also be utilized to assess global and regional LV systolic function and blood flow, which have been shown to be related to risk factors and prognosis (16-17). Relevant background data from sources other than the Strong Heart Study or the Cornell Echocardiography Laboratory are presented in this section.

1.2.1   Distribution of Measures of LV Size in Populations and Differences by Race

The best available information concerning the distribution of measures of LV size and mass has been provided by the Framingham Heart Study. Of a total of 6,148 participants studied by 2-dimensionally (2-D) guided M-mode echocardiography 80% had technically satisfactory LV measurements; this proportion exceeded 90% in subjects under 50 years of age but fell to 52% in those 70-79, and was also diminished by chronic lung disease and male gender but not consistently by obesity (18-19). In this population, the distribution of LV mass values appeared to be unimodal but skewed toward higher values, whereas in the subset (14% of subjects) who were considered to be normal, LV mass was normally distributed (i.e., demonstrated a bell-shaped curve) (18, 20). In the entire population LV mass rose steeply with age (21), but among apparently healthy subjects LV

mass increased slightly in older women but actually fell among men (22). The difference in the distribution of LV mass between the entire population and the normal subset reflected the relatively high levels of LV mass in individuals with hypertension, obesity, and prevalent coronary and valvular heart disease and the progressive increase in the prevalence of these conditions with advancing age (21). Only in apparently healthy young men was a weak positive relation of physical activity to LV mass observed (23). Although few non-white subjects were studied in Framingham, other echocardiographic studies have revealed racial differences in cardiac structure and function. Thus, Dunn et al (24) found higher LV mass in black than white hypertensives with similar blood pressure, and Soto et al (25) found higher peripheral resistances in black than white adolescents in Bogalusa. These differences have been confirmed by Liebson et al (26) who found significantly higher LV wall thicknesses in 173 black than in 671 non-black participants in the Treatment of Mild Hypertension Study. No comparable data are currently available concerning LV size, mass, or function in Native Americans.

1.2.2   Association of LV Mass and Function to Prevalent Heart Disease and Subsequent Morbidity

Although numerous clinical studies have documented associations between LV mass and both risk factors (e.g., obesity and hypertension) and overt coronary, valvular or myocardial heart disease, these studies suffer from the common limitation of difficulty in determining the impact of referral patterns and subject selection criteria on the observed results. Framingham and the ongoing Cardiovascular Health Study of subjects ≥ 65 years of age will provide increasingly complete data concerning these associations for Caucasian subjects (who comprise about 99% and >90%, respectively, of participants) but no comparable data are yet available for American Indians. This is unfortunate because known divergences of patterns observed among American Indians from those in Caucasians (e.g., high prevalences of diabetes and obesity but low cardiovascular disease rates among Pima Indians [27]) suggest that study of this group may clarify whether the emerging pattern of risk factor-preclinical disease (e.g., LV hypertrophy) relations are equally observed in groups with varied genetic and risk factor characteristics or are relatively specific to urbanized Caucasian populations.

Echocardiographic LV mass has been related to subsequent morbidity and mortality in both epidemiologic and clinical studies. Again, the most generalizable data come from Framingham. Levy and colleagues (3, 13) demonstrated that baseline LV mass was a strong predictor of cardiovascular morbid and mortal events and all-cause mortality during 4-year follow-up, and indeed that "only LV mass and age demonstrated consistent and strong relations to all three outcome measures" (3). This predictive value of LV mass has been confirmed in several clinical studies of Caucasian and Black patients (11-12, 14-15, 28), but no data are available concerning Native Americans.

1.2.3 Obesity and LV Size

Although much of the adverse effect of obesity on health is mediated through its effects on blood pressure and lipid profile, large-scale studies have demonstrated an independent relation between obesity and cardiovascular risk (29-30). Clinical studies (31-33) and data from Framingham (21) have shown positive relations between obesity and increased LV mass and have been taken to suggest that the latter may contribute to the adverse effects of obesity. However, knowledge of the relation of increased body fat to LV mass has been limited because: 1) most studies have evaluated adiposity indirectly from body proportions by calculation of body mass index (BMI=([weight in kg]/[height in m]^2)); 2) the increase in lean body mass commonly found in subjects with high BMI has generally not been taken into account; and 3) the correct method of relating LV mass to body size has been controversial, with indexation for body surface area potentially being too forgiving of obesity (20) while implicit to the alternative method of indexing LV mass by dividing it by body height is the assumption that the 3-D volume of an organ such as the heart should be linearly related to the one-dimensional measurement of height (34). To resolve these questions, data are needed in which measurements of adipose and lean body mass are related to LV mass free of the constraining assumptions implicit in conventional methods of indexation.

1.2.4 What are the cardiac functional characteristics and prevalence of the cardiomyopathy of diabetes and can its early features be detected in individuals with impaired glucose tolerance?

Clinically overt diabetes mellitus is a potent risk factor both for atherosclerotic cardiovascular disease and for congestive heart failure without myocardial infarction. A recent pathologic study has documented cardiac abnormalities related to diabetes in post-mortem human hearts that were independent and additive to those associated with hypertension (35). Only limited data on the in vivo effects of spontaneous diabetes per se on the heart are available, due in part to 1) the low prevalence of type I ("juvenile") diabetes in the young general population, precluding assembly of adequate-sized groups of young diabetics; and 2) the relatively old age at onset and strong association between type II diabetes and large-vessel coronary artery disease in Caucasian populations, making the cause of observed cardiac abnormalities uncertain unless subjects had undergone coronary arteriography. The high prevalence of type II diabetes with a relatively young age of onset (36) and modest prevalence and incidence of coronary artery disease among diabetics (37) in full-blooded American Indians, especially the Pima, makes this ethnic group an attractive one in which to study non-coronary diabetic heart disease.

1.2.5 Are circulating insulin levels associated with the level of left ventricular mass, independent of conventional stimuli to cardiac growth?

The relatively weak relations between blood pressure and body build, on the one

hand, and LV mass on the other have often been taken as indirect evidence of non-hemodynamic regulation of growth of cardiac and vascular muscle (38). Although the level of volume load (i.e., the amount of blood pumped) has recently been recognized as an additional hemodynamic stimulus to increased LV mass (39-40), increasing attention is being paid to potential growth factors. Among the latter insulin merits particular consideration in view of emerging associations of glucose intolerance and insulin resistance with a spectrum of cardiovascular abnormalities (16, 41). The only available study relating insulin resistance to LV mass initially appeared to be negative (42) but had turned positive as additional cases were added before the time (11/90) the abstract was presented. The very high prevalence of abnormalities of glucose and insulin metabolism among the Pima and more moderate prevalences of these abnormalities among other Native American groups makes Strong Heart Study subjects an attractive group in which to study insulin-LV mass relations.

1.2.6 Is increased alcohol intake associated with left ventricular hypertrophy and at what level of intake does ventricular dysfunction become evident?

Although modest ethanol intake may have beneficial effects on lipid profile and cardiovascular morbidity, excess alcohol use leads to accelerated rates of cardiac death (43). This may be mediated by an alcoholic cardiomyopathy which is at least partially reversed by abstention. An association between alcohol intake and LV mass was reported by Manolio et al (44) based on Framingham data. To date no population-based assessment of the relation of alcohol intake to measures of LV function has been reported. The relatively high rate of excess alcohol assumption among Native Americans makes them a population in which it is practical to study alcohol-heart relationships with an adequate sample size.

1.2.7 What is the prevalence of and factors associated with valvular heart disease in Native Americans?

While the burden of cardiovascular disease is generally thought of in terms of coronary heart disease, it is notable that cardiac valvular surgery is undertaken annually in nearly 30,000 Americans. Reliable data on the population prevalence of significant valvular stenoses and regurgitation are not available, but are likely to emerge soon for Caucasian populations from Framingham and the CHS. Although Native Americans have commonly had limited health care, which may predispose to rheumatic fever, and have high prevalences of obesity, which appears to be associated with valvular degeneration, no data are available on the prevalence, severity, or factors associated with valvular heart disease in this population.

1.3 Preliminary Studies

Findings and concepts relevant to the specific questions are discussed separately

although it is recognized that the divisions are in part artificial.

1.3.1   What is the distribution of measures of LV mass, size and function among Native Americans and does it differ from that found in Caucasians?

The Cornell laboratory has extensively validated and standardized echocardiographic measures of LV size, mass, function and hemodynamics (9, 45-53). Study of a multi-ethnic employed population in New York City revealed unimodal distributions of LV mass, LV internal dimension and wall thicknesses among normotensive adults whereas among sustained hypertensives the distribution of LV mass appeared to be bimodal with a second, higher made at about 120 g/m^2 (54). In this employed population LV mass was independently related to height, systolic blood pressure, body mass index, and gender (55). In the employed hypertensives and in a separate clinical population of hypertensive patients, LV wall thicknesses and mass were higher among blacks than whites (54, 56-57). This racial difference may also have contributed to slightly higher LV masses in the multi-racial Cornell normotensive group than in the overwhelmingly Caucasian normal groups studied in Framingham (18) or in Naples, Italy (58).

Left ventricular fractional shortening and fractional shortening adjusted for LV end-systolic stress also were normally distributed in the Cornell normotensives, whereas stress-adjusted fractional shortening had a bimodal distribution among hypertensives suggesting enhanced function in a subset (59). Other studies supported the usefulness of stress-adjusted fractional shortening as a simple measure of contractility (51, 60) but also suggested that this measure is partially preload sensitive (61).

Although no preliminary echocardiographic data are available on SHS participants, indirect estimates can be made from ECG findings. Physician readings of the first 1810 ECGs yielded diagnoses of LV hypertrophy in 2.2% of the population. This prevalence resembles that found by standard ECG criteria in Framingham (2.1%) (62). In view of the low sensitivity of the ECG in both Framingham (7%) and among employed hypertensives in New York (9%) and the diminished sensitivity of the ECG for increased LV mass in obese subjects (63) this suggests that the prevalence of increased LV mass in SHS subjects will approximate the 12 to 19% found in Framingham and at Cornell. The proportion of SHS subjects with increased LV mass may be even higher, however, because the presence of obesity may mask ECG recognition of LV hypertrophy in may participants.

1.3.2   What is the association of left ventricular mass and functional measures to prevalent clinical heart disease and subsequent incident mortality and morbidity?

The Cornell investigators have extensively studied the prevalence of LV hypertrophy and dysfunction in patients with various forms of cardiovascular disease. Among hypertensives, the proportion with LV mass above sex-specific upper normal limits ranged from 12 to 19% among healthy employed adults (54) to 44% among outpatients at a

referral hypertension center (47, 49) to 87% among patients hospitalized for relatively severe hypertension (47, 49). LV mass was even more consistently increased among patients with hemodynamically important aortic regurgitation (93% [64]), mitral regurgitation (84% [65]), and dilated cardiomyopathy (68% [49, 51]). LV dysfunction has been shown to be rare at rest (but relatively common during exercise [66]) in asymptomatic hypertensives, whereas it is relatively common in other forms of heart disease. Data for specific heart diseases from the SHS clinical examination reveal high prevalences of hypertension (19 to 60% among men and women with different levels of glucose tolerance in the 3 SHS regions) and at least suggestive evidence of coronary heart disease in nearly 20 percent of participants.

Research from Cornell, from Framingham and from a largely African-American population in Chicago has demonstrated the prognostic significance of echocardiographic LV mass. In initially uncomplicated essential hypertensive patients, baseline echocardiographic LV mass measurements were a stronger predictor of cardiovascular events during 10-year follow-up than any other variable except age (4). In fact, entry of age and LV mass into multivariate analyses eliminated the predictive value for morbid events or death found in univariate analyses for conventional risk factors (e.g., systolic blood pressure, cholesterol) (4). Of note, the predictive value of LV mass was greatest for cardiovascular and all-cause mortality, which would be feasible to ascertain in the future by national death Index data whether or not the SHS is continued after the present funding period. However, the Framingham and New York populations exhibit similar relations between conventional risk factors and cardiovascular events. In contrast, these relations are dramatically weaker in the Pima, who exhibit high prevalences of obesity and diabetes but low ones of clinical and ECG myocardial infarction, and to a lesser extent among other participants in the SHS. Thus, assessment of the predictive value of LV mass for subsequent events in the SHS can help determine whether or not the LV mass-morbid event relation is a fundamental one that is independent of genetic background and the mix of concomitant risk factors.

1.3.3   Is increased body fat a major stimulus to LV enlargement and hypertrophy independent of the effects of lean body mass or blood pressure?

Previous research at Cornell has demonstrated increases in LV mass and variable chamber enlargement in overweight as opposed to normal-weight hypertensive and normotensive adults (55, 58). Other analyses showed that the well-known gender difference in LV mass was proportional to differences in skeletal muscle mass estimated from 24-hour urinary creatine excretion (45), and that obese, hypertensive men also had high skeletal-muscle masses (55). To further evaluate the relations between body proportions and LV mass, we compared echocardiographic and body habitus variables in 611 normal weight and 56 overweight-to-obese adults and children studied at Cornell and in Naples, Italy and Cincinnati, Ohio (58). In normal-weight subjects, LV mass was linearly related to body weight, but increased disproportionately with increases in body

surface area (to approximately its 1.5 power) or height (to its 2.7 power). LV mass was higher in overweight subjects but by smaller percentages than the increases in body weight or surface area, thus causing LV mass as a ratio to these measures to be reduced in overweight subjects; indexation of LV mass/height$^{2.7}$ appeared to do slightly better than indexation by height in identifying the increase of about 14% in LV mass from values found in normal-weight subjects of the same height (58).

The SHS presents advantages for clarification of the impact of adiposity on heart size compared to existing data: 1) adipose body mass is estimated directly by bioelectric impedance (67) and 2) a higher prevalence of overweight (mean body mass index about 31 kl/m^2 with about 60% of subjects above upper normal limits) than of hypertension facilities separation of these two stimuli to myocardial growth.

1.3.4 What are the cardiac functional characteristics and prevalence of the cardiomyopathy of diabetes, and can its early features be detected in individuals with impaired glucose tolerance?

Diabetes has been an exclusion factor from normotensive and hypertensive populations studied by echocardio-graphy at Cornell, with one small exception (68). A number of studies at Cornell have characterized the distribution of LV systolic function indices and their relation to myocardial afterload (end-systolic stress) in non-diabetic normotensive and hypertensive populations and patients with other forms of heart disease (51, 59, 66, 69-70). Results from the first SHS clinical examination indicate that sufficiently large numbers of subjects should be available for echocardiographic study in each of the 18 gender (male/female)-region (Arizona-Oklahoma-Dakota)-glucose metabolism (normal-impaired-diabetic) cells in the SHS to facilitate analyses comparing LV findings in subjects stratified by level of glucose intolerance with the capacity to control for relevant confounding variables.

1.3.5 Are circulating insulin levels associated with the level of LV mass, independent of conventional stimuli to cardiac growth?

This question has not been addressed at Cornell, or in other large-scale studies. Plasma insulin measurements obtained during the ongoing SHS clinical examination indicate that levels vary widely within and between gender-region-glucose tolerance cells, with particularly extensive overlap between subjects with normal and impaired glucose tolerance. About 1,550 SHS subjects were classified as having normal glucose tolerance, 750 as having impaired glucose tolerance, and about 2200 as having overt diabetes on the first clinical examination, providing reasonably large subject groups in which to evaluate associations of insulin levels and insulin-glucose relations to LV findings.

1.3.6 Is increased alcohol intake associated with LV hypertrophy and at what level of intake does LV dysfunction become evident?

Recognized alcoholism has also been an exclusion criteria from Cornell studies. In SHS data obtained to date, approximately 15 to 25% of women and 30 to 40% of men were classified as regular drinkers and 10 to 20% of women and 20 to 50% of men as binge drinkers. Thus, exposure to alcohol is substantial, but not uniform, in SHS participants.

1.3.7 What are the prevalence of and factors associated with valvular heart disease among Native Americans?

The Cornell laboratory has performed extensive research on echocardiographic methods to detect, identify the etiology and characterize the severity and reversibility of valvular heart diseases (64-65, 71-77). Other studies have estimated the prevalence of mitral valve prolapse at about 4% in employed subjects and clinical populations (78) and have used case-control methodology to assess the associations between mitral prolapse and infective endocarditis or severe mitral regurgitation (78-80). This extensive research experience gives the Cornell investigators a high level of expertise in detecting common forms of congenital, rheumatic and degenerative valvular heart disease.

1.4 Methods of Echocardiogram Performance, Interpretation and Analysis

The echocardiographic procedures for the Strong Heart Study have been designed with particular regard to the special difficulties of performing objective, skill-dependent cardiac tests in several variably remote sites. Procedures are presented in general outline followed by detailed description of 5 principal segments: 1) equipment to be used; 2) initial training/start-up; 3) echocardiography performance at Field Centers; 4) central coordination and echocardiogram reading at The New York Hospital-Cornell Medical Center; and 5) wrap-up/data analysis.

1.4.1 General Outline:

Echocardiograms will be performed over a 27 month period beginning in August 1993 on participants aged 47 to 78 in the Strong Heart Study (SHS). Study echocardiograms will be performed on the approximately 4,050 SHS participants returning for exam II, in addition to duplicate echocardiograms on about 200 subjects to assess reproducibility and potentially on a small number of additional subjects who were eligible for but did not participate in SHS exam I. To perform this number of echocardiograms with appropriate quality-control procedures will require one or two sonographers for each SHS region (Arizona, Oklahoma and the Dakotas). During the lead-in/training period from February through June 1993 (and thereafter as necessitated by logistical difficulties for the Dakota center) sonographers will be selected by Field and Reading Center investigators and receive initial training locally followed by intensive training in the specific study protocol at the Echocardiography Reading Center in New York.

Standardized examinations will include 2-D guided M-mode echocardiograms and selected 2-D and Doppler recordings. Studies will be sent to New York for blinded interpretation by experienced technician and physician readers. Study performance and interpretation will focus on selected measures of LV mass and geometry, global and regional systolic function and diastolic filling to maximize the yield of reliable data to answer the 7 specific questions.

Because of the long distances involved, multiple steps are planned to maximize quality control of echocardiogram performance. These include performance of preliminary measurements of LV dimensions and other variables by the examining technicians, using a standard form (Appendix I). This will increase their awareness of aspects of image orientation and definition needed for a measurable study. A copy of videotaped 2-D and M-mode views on study subjects will be returned to the Field Centers from the Reading Center with final measurements and comments and suggestions about how to enhance technical quality, for continuing education of the field technicians. Periodic site visits to Field Centers will be made by Reading Center staff. The Reading Center will use procedures adapted from those developed and refined in the Cornell laboratory over the past 12 years. Steps to assure data quality will include blinded performance of measurements, checking of initial measurements against the visual appearance of the echocardiograms, verification of technician-reader measurements by experienced investigators, and repeat verification of all measurements that fall outside expected ranges for a normal to mildly diseased population or reveal unexpected relations among variables. Computer support and assistance with data management and statistical analyses will be provided by the computer Center of the General Clinical Research Center and by the Division of biostatistics at Cornell.

1.4.2 Equipment

Echocardiograms will be performed using Acuson 128 echocardiographs equipped with 2.5/3.5 megahertz and 2.0 megaHz probes. These echocardiographs have been previously used successfully in the multi-center NHLBI-sponsored CARDIA study. The four echocardiographs will be assigned, one each to Arizona and Oklahoma and two to the Dakotas because of the long distances between study sites.

For performance of the echocardiograms, the Acuson echocardiographs will be mounted in specially-designed vans that will also be used for pulmonary function testing. As shown in Figure 1, the vans will be equipped with examining tables designed to facilitate performance of standardized, quantifiable echocardiograms.

At the Reading Center quantitative and qualitative assessment of echocardiograms will be performed using a Digisonics Cardiorevue Center.
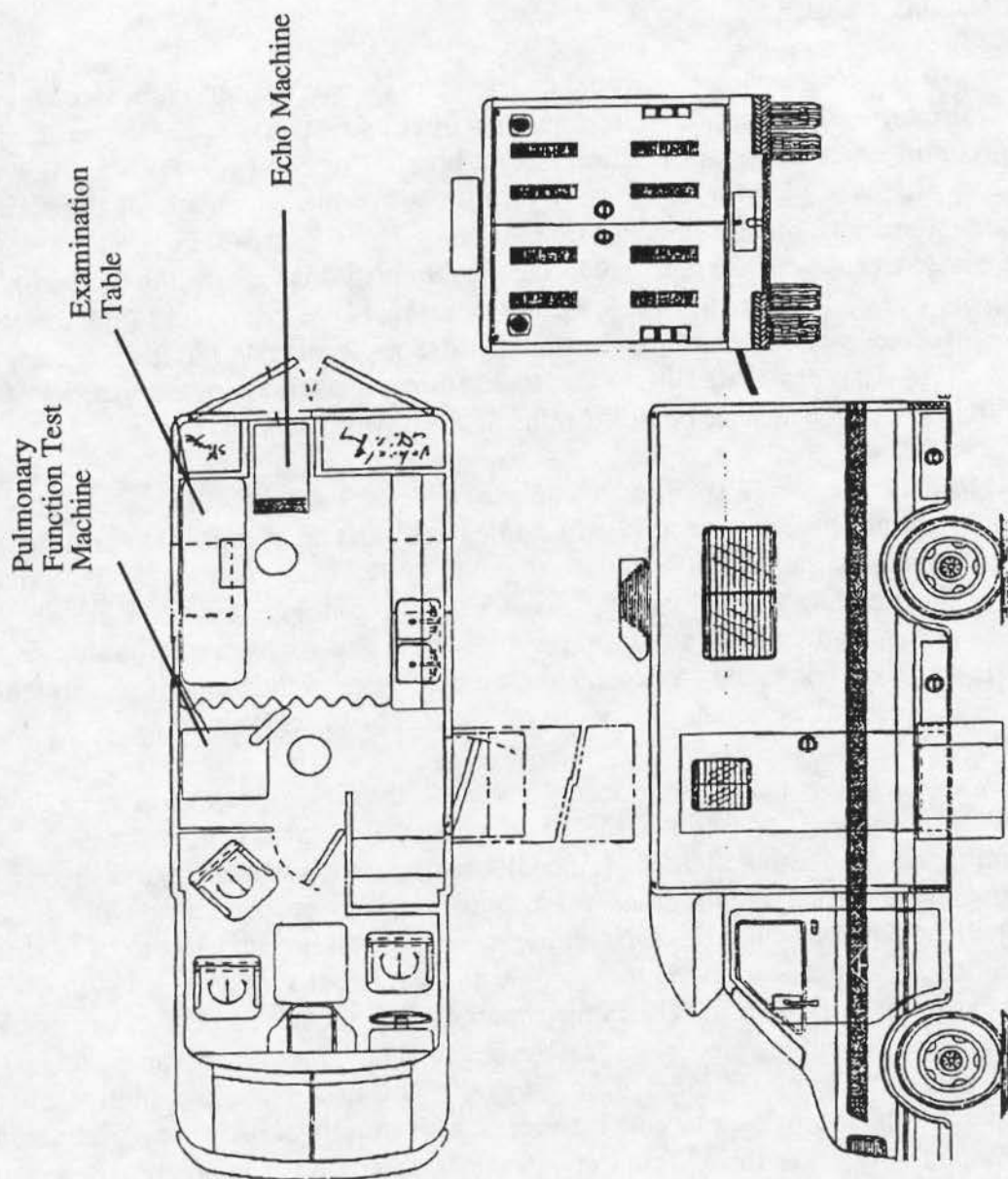
Figure 1. Floor plan and external view of echocardiography/pulmonary function test vans for the Strong Heart Study.

1.4.3 Initial Training/Start-Up

Once selected, technicians would undergo phased training during the three months before the performance period. Pretraining will consist of a) supervised training as appropriate in echocardiogram performance in hospital or clinics in Phoenix, Lawton-Oklahoma City and elsewhere; b) study of an instructional videotape of the specific echocardiogram performance protocol for the Strong Heart Study (SHS); and c) study of other selected videotaped materials on echocardiogram performance. During this period the technicians will perform studies using locally available echocardiographs (and the study Acuson machines when available) following the SHS protocol and send the videotapes to New York by Express Mail for review and return of teaching comments, in effect constituting a "correspondence course" for the SHS study protocol.

Formal training will begin with a course in New York that will combine didactic teaching of selected general aspects of echocardiography and the specific SHS protocol and hands-on training in performing echocardiograms by the study protocol. The training course at the Reading Center will be two weeks for sonographers who are already proficient in echocardiography but not experienced with quantitative research studies. To maximize the degree of hands-on experience and the degree of individualized instruction, the training course will be conducted for one or two sonographers at a time.

After the sonographers return from New York, the Field Centers will be equipped. This will include arrival and set-up of the 4 Strong Heart Study vans equipped with Acuson echocardiographs and installation of additional low-frequency (2 megaHz) transducers on these machines. Final preparations for echocardiogram performance will also be completed, including installation of examining-table mattresses with cut-outs to facilitate access to the apical echocardiographic window and screens/shades needed to allow dim lighting for study performance. The camper trucks will be equipped with an appropriate examining table, fastening systems (both at the base and upper portion of the machines) to keep the echocardiograph in place during travel. This will provide mobility among scattered sites in South Dakota and between the examining centers in Arizona and Oklahoma and also make the addition of echocardiography possible in centers without available space for this procedure. Two units will be used in South Dakota, one at Pine Ridge and the other shared between Eagle Butte and Ft. Totten since the communities are so far apart. Oklahoma and Arizona will use one each.

With the equipment in place, a pilot/on-site-training study will be performed in each region during July 1993, after the Black Hills training course for all SHS employees. During a 2-day site visit by a member of the Reading Center staff each technician will perform echocardiograms on 4-6 subjects each day. This will permit immediate feedback to complete supervised technician training in the SHS protocol, and will also provide small-scale data on inter-technician variability. Between the training course in New York and the beginning of the performance period, technicians will perform studies on volunteers, make

preliminary measurements, and send the echocardiograms and interpretations to New York for review and feedback.

It is the principal investigator's experience over the past 15 years that the percentage of echocardiograms that are suitable for accurate measurement is enhanced if the examining sonographer makes preliminary measurements on each study and is then provided feedback as to how to improve the suitability of the study for quantitation and the selection of interfaces to measure. To obtain reproducible M-mode measurements of LV structures dominant lines representing the necessary interfaces should be recorded, and recognized during interpretation,that exhibit continuous motion in the correct pattern for the structure for at least 0.10 second but ideally through the entire cardiac cycle (6, 48, 81).

1.4.4 Echocardiography Performance at Field Centers:

Principles: The most important primary echocardiographic measurements and derived variables to assess the heart in an epidemiologic context can be obtained from a relatively simple echo examination (50, 82). Correct orientation of the ultrasound beam and imaging planes to LV structure and blood flow is essential.

The LV resembles an ellipse of rotation that is nearly circular in short-axis views, with a long-axis about twice its minor axis. To measure the LV minor axis accurately it is necessary to orient the echocardiographic beam from the parasternal (or less commonly the subcostal) window to pass perpendicularly through the interventricular septum and posterolateral LV wall at the junction of papillary muscle tips and mitral chordae under 2-D guidance (Figure 2A). Rotation of the 2-D sector 90° to the short axis projection allows one to measure the true, maximum LV diameter (Figure 2B). If, as is common in older subjects, the best parasternal window is in a low interspace, LV minor-axis dimensions and wall thicknesses should not be measured in the usual fashion, although it may be possible to measure correctly the aortic root and left atrium (Figure 2C). Instead, a higher interspace should be used, which may image only a narrow sector that includes the LV minor axis (Figure 2D). If this is not possible, linear measurements of LV minor axis and wall thicknesses should be made at the correct level and orientation by the leading-edge method from 2-D long-axis views that maximize LV cavity size.

M-mode LV recordings taken from a low window can commonly be recognized by the appearance in systole of additional echo interfaces along the left side of the interventricular septum that was not seen during diastole (Figure 3). This occurs because segments of the septum closer to the LV base are drawn into view of the M-mode beam by LV contraction.
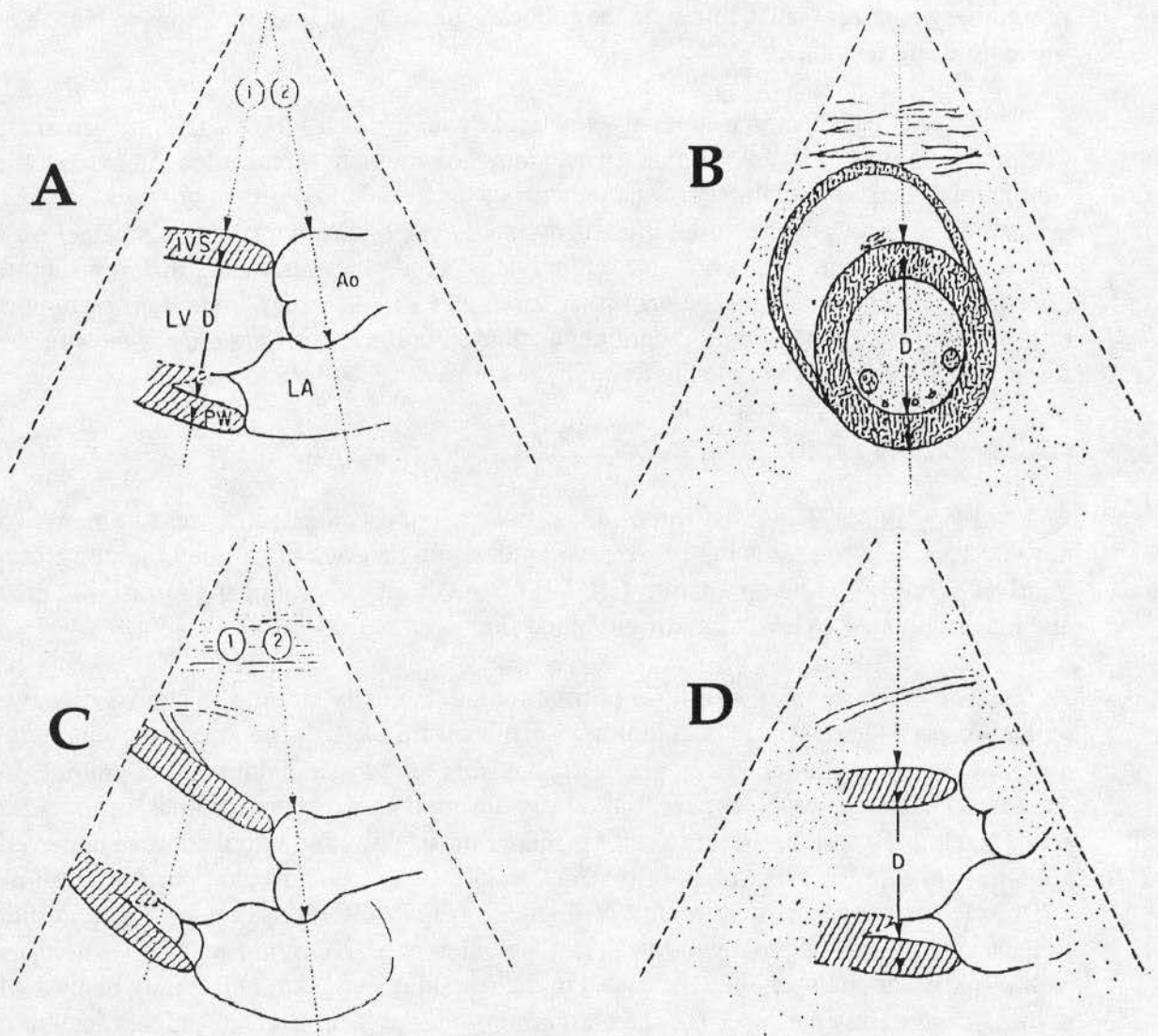
Figure 2. Orientation of parasternal long- and short-axis two dimensional echocardiographic imaging planes. **A:** Long-axis view in which the M-mode cursor would be correctly oriented to both the left ventricle and the aorta and left atrium. **B:** Rotation of the transducer approximately 90⁰ results in an optimally oriented short-axis view. **C:** Long-axis view from a lower parasternal window in which the M-mode beam or short axis tomographic plane is obliquely oriented to the left ventricle, as is common in older individuals. **D:** Movement of the transducer one interspace higher than used for part C permits correctly oriented views of the left ventricle, albeit with a narrower field of vision.
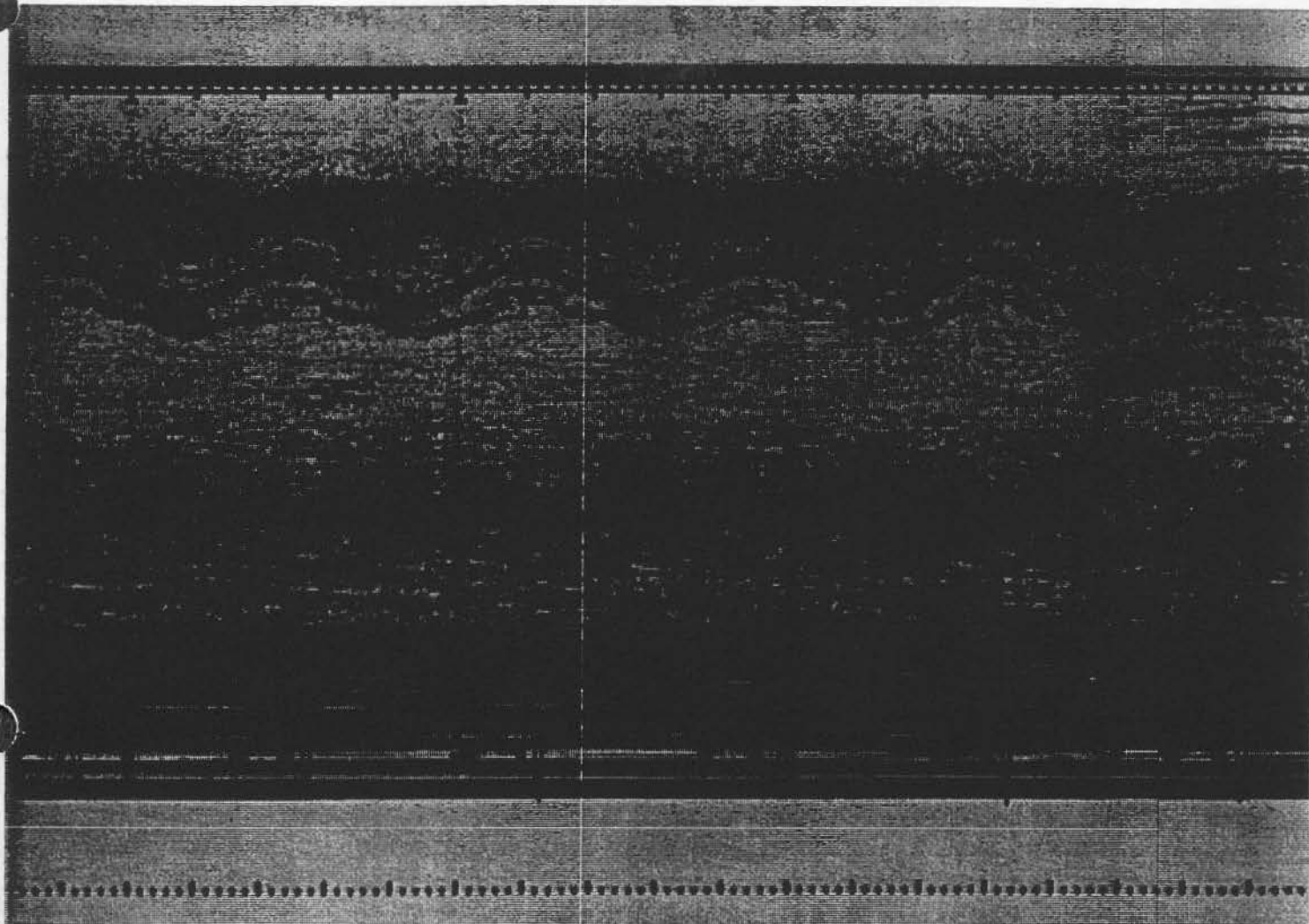
Figure 3
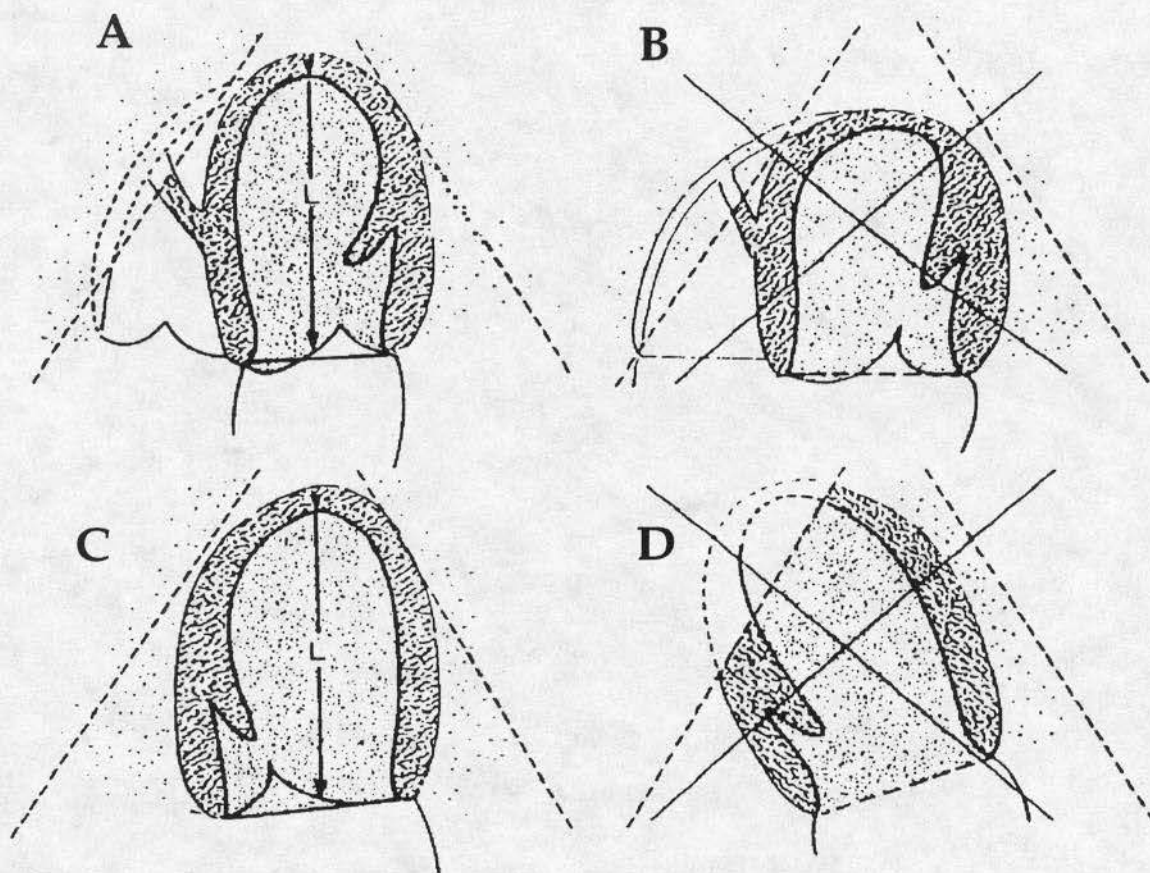
Figure 4. Orientation of apical four- and two- chamber two-dimensional echocardiographic views. In optimally oriented views the left ventricular apex is centered at the top of the sector "fan"in both four-chamber (**A**) and two-chamber (**B**) projections. The left ventricular long-axis is commonly foreshortened in the four chamber view (**C**), as is demonstrated by protrusion of the ventricular apex out of the field of vision in the two-chamber view (**D**).

A major advantage of 2-D echocardiography is its ability to visualize the LV long-axis and wall segments near the apex. To accomplish this, one must obtain the true (longest) long-axis dimension and visualize the LV walls in approximately orthogonal apical 4- and 2-chamber views. The LV long-axis is commonly foreshortened in the 4-chamber view (Figure 4A), as seen when the transducer is rotated to the 2-chamber view and the LV apex is out of the field of view (Figure 4B). The transducer should then be moved inferolaterally until the LV apex is as nearly centered at the top of the image "fan" in both views as possible (Figures 4C and 4D).

The accuracy of Doppler recordings depends on the ultrasound beam being parallel to the axis of blood flow. Variants of the apical 2- and 4-chamber views should be used to sample LV inflow across the mitral anulus or valve orifice while the apical long-axis view is best to measure systolic flow across the aortic anulus to calculate stroke volume and cardiac output.

1.4.5   Protocol for Echocardiogram Performance

Standardized methods will be employed to obtain high-quality recordings. Echocardiograms will be performed in specialized vans with an area that provides room for the examining table, echocardiograph, etc., and have dimmable lighting to prevent glare on the echocardiograph screen that would interfere with study performance. Subjects will change their top for a light gown to permit discrete exposure of the chest wall overlying the parasternal and apical acoustic windows. Disposable ECG lead attachments (set of 3) will be attached to the skin to monitor a single ECG lead for timing purposes. The subject will then lie down and assume a partial left decubitus position (with pillows or a foam-rubber wedge to support the back) with the head of the examining table modestly elevated. The subjects' last name, initial, SHS study number and the date and site of recording will be entered so they will be recorded on videotape. Echocardiographic recordings will then be made using procedures outlined in Table 1. Careful performance of this protocol will require 40 minutes of subjects' time including the period required to get in and out of a gown and to step from the IHS facility to and from the adjacent van.

TABLE 1   ECHOCARDIOGRAPHIC TECHNIQUES FOR LEFT VENTRICULAR
MEASUREMENTS

Instrument Calibration:	Calibrate against phantom at installation and at regular intervals thereafter.
Echocardiographic Performance:	Standardize and record decubitus position. Use mattress cut-out for apical imaging. Record images in held expiration.
Location of Imaging Planes:	
2-Dimensionally guided M-mode:	From short-axis view with correct angulation of short-axis plane defined in long-axis view or in long axis with maximization of left ventricular cavity diameter.
2-Dimensional Echo:	Define correct orientation of short-axis and apical views by use of 90 degree orthogonal planes.
Recognition of Measurable Images:	
M-mode:	Dominant lines with correct motion representing interfaces for at least 0.10 seconds (5 m m a t standard recording speed).
2-Dimensional Echo:	Visualization of complete interface in motion with persistence in stop-frame mode.
Enhancement of Reproducibility:	
Use three or more cardiac cycles.	
Record imaging window location and patient position.	
For research use readings by two or three investigators.	

Modified from Devereux et al (reference 50).

1.4.6   Specific recordings will be made as outlined in Table 2 and the following text:

Parasternal Long-Axis 2-D recordings will be obtained first, with the interspace and degree of left decubitus positioning chosen to allow an M-mode cursor line to traverse the interventricular septum (IVS) and LV posterior wall (PW) perpendicularly.

TABLE 2   STRONG HEART STUDY ECHO/DOPPLER SCANNING AND RECORDING
                            SEQUENCE

I.   <u>Parasternal Long-Axis Orientation View</u>

     A.   Two-dimensional echocardiography during quiet respiration: Maximize left ventricular and aortic diameter and record 10 beats on tape.

II.   <u>Left Ventricular Imaging</u>:

     A.   M-mode cursor perpendicular through left ventricle just below the level of the mitral leaflet tips: Record 10 beats of 2-D update image with M-mode recording, then record at least 10 beats of full-screen M-mode during quiet respiration and attempt at least 5 beats at held-expiration; finally, record a full-screen, freeze-frame M-mode image for 5 seconds at held-expiration.

     B.   Turn 90° into parasternal short-axis view.

     C.   Two-dimensional echocardiography at or just above level of papillary muscle tips during quiet respiration: Record 15 beats on tape.

     D.   M-mode cursor through the meridian of the left ventricle at level of papillary muscles: Record 10 beats of 2-D update image with M-mode recording and 10 beats of full-screen M-mode recording during quiet respiration on tape. Then attempt at least 5 beats at held-expiration; finally, record a full-screen, freeze-fame M-mode image for 5 seconds at held-expiration.

III.   <u>M-mode sweep</u> from LV through mitral valve to left atrium/aortic view recorded on videotape.

IV.   <u>Aortic Left Atrial Imaging</u>:

     A.   Two-dimensional echocardiography in long-axis views during quiet respiration at level of aorta and left atrium with maximization of aortic diameter at the sinuses of Valsalva: 10 beats.

     B.   M-mode cursor perpendicular through aorta and left atrium with maximization of aortic diameter by "tilting" medially and laterally of the 2-D imaging plane: Record 10 beats of 2-D update image with M-mode recording, then record 10 beats of full-screen M-mode during quiet respiration; finally, record a full-screen, freeze-frame M-mode image for 5 seconds at held-expiration.

C. Color Doppler will be turned on to record 10 beats of a view encompassing the left ventricular outflow tract and left atrium.

V. Apical Four-Chamber View

A. Two-dimensional echocardiography in quiet respiration. Record at least 10 beats with maximum chamber dimensions and good LV endocardial definition on tape.

B. Pulsed Doppler transmitral flow recording with sample volume at the mitral anulus leaflet tips during diastole: Using a 2.5 MHz transducer, record 10 beats of 2-D update image with Doppler recording, then record 10 beats of full-screen Doppler during quiet respiration.

C. Doppler color flow mapping during quiet respiration: Using the 2.5 MHz transducer, turn on color to look for mitral regurgitation: Record 15 beats on tape. while sweeping from the 4- to the 5-chamber view.

D. Turn approximately 90° into apical two-chamber view.

VI. Apical Two-Chamber and Apical Long-Axis Views

A. Two-dimensional echocardiography in the true apical two-chamber view during quiet respiration or held expiration: Record 15 beats with maximum LV chamber dimensions and good LV endocardial definition on tape.

B. Two-dimensional echocardiography in the apical long-axis view during quiet respiration or held expiration: Record 10 beats taking care to include the left ventricle, left atrium, aorta and right ventricle in the image.

C. Pulse Doppler recording in the apical long-axis view: Record 10 beats of 2-D update image with pulsed Doppler recording at the plane of the aortic valve anulus (hinging points of the aortic cusps), then record 10 beats of full-screen Doppler during quiet respiration.

Left Ventricular Imaging: While recording on super VHS tape, the imaging plane will be tilted medially and laterally to maximize the LV cavity area in the long-axis view. The M-mode cursor line will than be optimally oriented in the 2-D long view just basal to the level of the papillary muscle tips; at least 10 cycles of LV M-mode recordings with 2-D update and a second 10 cycles of full-screen M-mode will be made on videotape. If feasible, 5 cycles of full-screen M-mode will be recorded in held expiration and a full screen freeze-frame will be recorded on videotape. If another imaging window is subsequently recognized to be superior, the orientation and full-screen M-mode recordings

during quiet respiration will be repeated. A second 10-cycle M-mode LV recording will be made on 20% of subjects for use in assessment of measurement reproducibility. An attempt will be made to include a period of held expiration in LV recordings unless this interferes with LV visualization. The 2-D imaging plane will be rotated from the chest wall position that permitted optimal long-axis M-mode cursor orientation, approximately 90 degrees to visualize the LV short-axis view, at or just towards the LV base form the visible landmark of the papillary muscle tips. Recordings will then be made as indicated in Table 2.

An M-mode "sweep" will then be made from the LV through the mitral valve to the aorta/left atrium level. At the aorta/left atrium level, 2-D long-axis recording will be resumed, the imaging plane will be manipulated to maximize aortic diameter, the cursor beam will be oriented through the sinuses of Valsalva at their maximum diameter and M-mode recordings will be made as described in Table 2. Color Doppler will be turned on to record 10 beats of a view encompassing the LV outflow tract and left atrium.

At the completion of these recordings the transducer will be shifted to the apical window, identified by palpating the location of the LV impulse on the chest wall and then moving the transducer inferolaterally until the LV apex is visualized in both 2- and 4-chamber views. Repositioning of subjects may be needed to obtain a good apical acoustic window. When this is accomplished, the 2- and 4-chamber views that maximize LV cavity size will be recorded (at least 10 cycles of each); in the 4-chamber view pulsed Doppler recordings of blood flow velocity at the mitral anulus will be recorded (Figure 5A)(10 cycles) and then color Doppler will be turned on for another 15 cycles as the imaging plane is swept from the 4- to 5-chamber view. After completion of these recordings, the transducer will be rotated to the apical long-axis view (which visualizes the aortic valve and root as opposed to the 2-chamber view which excludes them in favor of the anterior LV wall) and pulsed Doppler recordings of blood flow at the aortic anular plane (10 cycles) will be performed (Figure 5B).
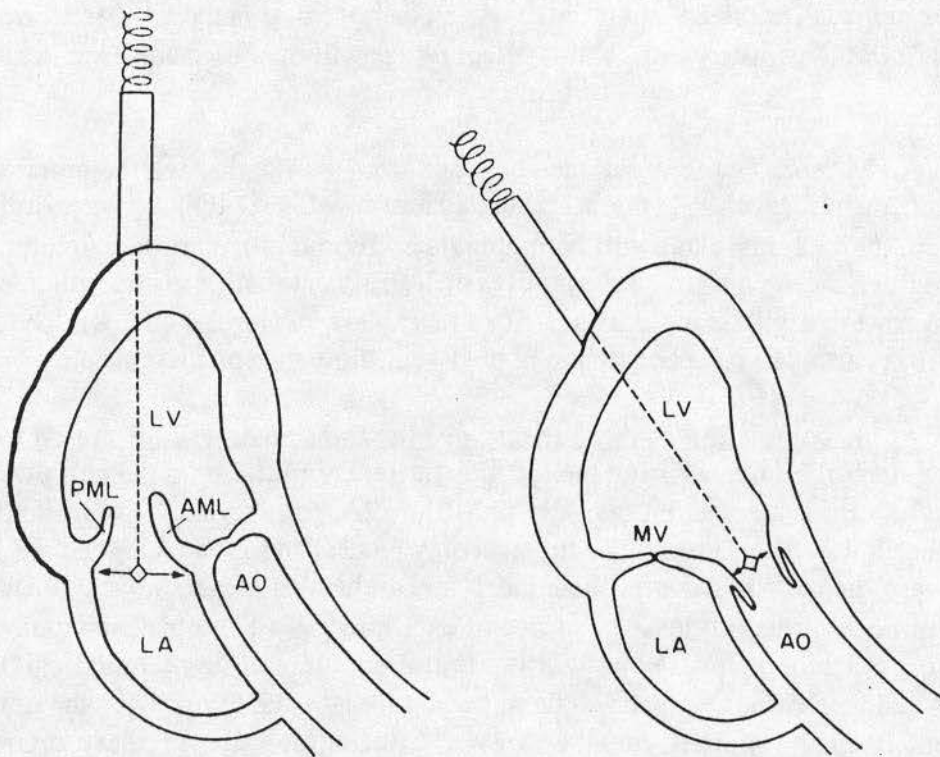
Figure 5. A: Schematic diagram showing the location of pulsed doppler sample volume for evaluation of left ventricular diastolic inflow at the level of the mitral valve orifice (◊) in an apical long-axis view oriented to maximize the diameter of the mitral annulus. B: Location of pulsed Doppler sample volume (◊) at the level of the aortic annulus in apical long-axis view. Abbreviations: AML, anterior mitral leaflet; Ao, aorta; LA, left atrium; LV, left ventricle; MV, mitral valve; PML, posterior mitral leaflet.
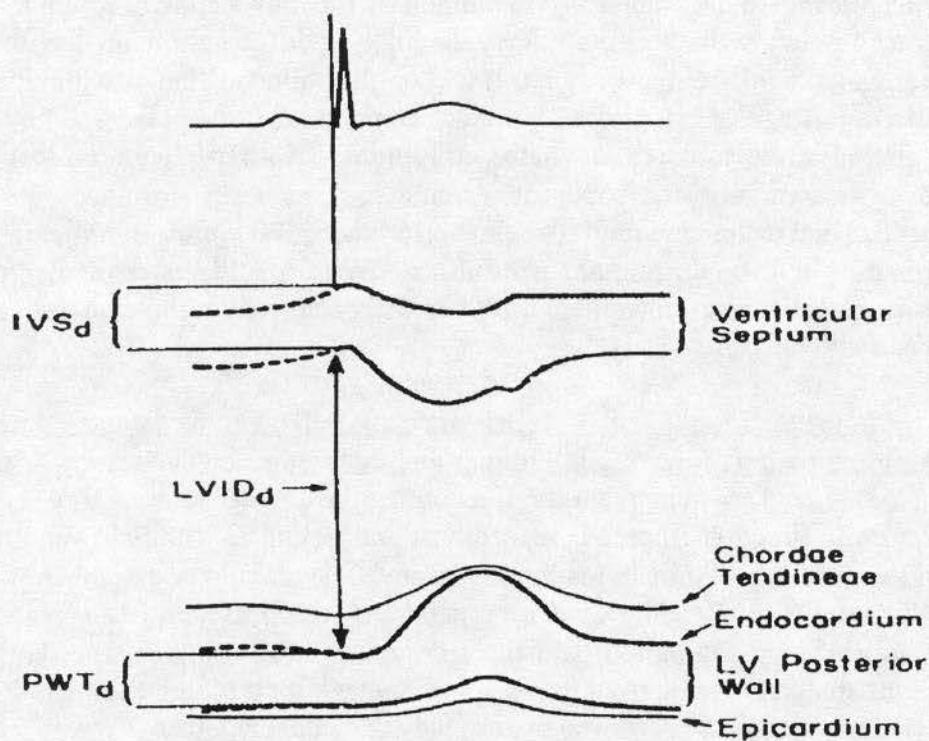
American Society of Echocardiography

Figure 6. Schematic depiction of M-mode echocardiographic left ventricular (LV) anatomic measurements according to the American Society of Echocardiography which recommended that end-diastolic measurements be made at the onset of the QRS complex using the leading edge of interfaces for all measurements.

At this point the subject will be returned to a supine position without turning up the lights or any other change and the blood pressure (phase 1 and 5 of the Korotkoff sounds=appearance and disappearance of sound) will be measured by appropriate-size cuff and mercury manometer. This is preferred to random-zero manometry because of documented inaccuracy of the latter (83). ECG leads will then be disconnected and the subject allowed to dress and to go to pulmonary function testing or return to the IHS clinic. The technician will then complete the logging information on the unblinded echo performance worksheet (Appendix 2) and on the "blinded" label (without identifiers that would reveal age, gender, blood pressure or body size) for videotape box (Appendix 3) that includes, the subject's last name and initial, SHS participant number, IHS number, date of performance and sonographer, and prepare the performance area for the next subject. Total technician time for echo performance (30 minutes), initial logging and area preparation will be 40 minutes per subject. With 6 subjects scheduled/day and slight inefficiency of subject flow, these activities will occupy the technician through the morning into early afternoon.

In the afternoon, Field Center technicians will continue the procedure begun during the training period of making preliminary measurements on each study of LV dimensions from 2-D guided M-mode recordings, recording the qualitative normality or abnormality of LV systolic function from 2-D recordings, and noting any clinical abnormalities. The worksheets with preliminary readings (Appendix I) will then be assembled with videotapes for shipment to the Reading Center, preparations (videocasettes, ECG electrodes, gel, etc.) for the next day completed, and the technicians will complete the day by reviewing previous studies returned from the Reading Center with teaching comments. Once a week, selected studies will be reviewed by the study personnel together.

1.4.7 Central Coordination and Echocardiogram Reading at The New York Hospital-Cornell Medical Center:

The Reading Center is responsible for design of the echocardiogram protocol, training and continuous feedback for quality control of echocardiogram performance by Field Center technicians, central reading of echocardiograms with careful procedures to assure accuracy and reproducibility of data, and on-going analyses (in appropriate conjunction with the Coordinating Center at the University of Oklahoma) to assure quality control and to test scientific hypotheses. The Center will take advantage of procedures and skills developed in this laboratory over the past 14 years in performing over 7,000 research echocardiograms in clinical patient groups, defined population samples, and large numbers of family units to study echocardiographic methodology (6, 9, 45, 49, 51), the heart in hypertension (11, 40, 46, 47, 59, 61, 66, 69-70, 84-87), heritable cardiovascular diseases (78-79, 88-95), valvular heart diseases (64-65, 72, 76-77, 96-98) and a variety of other conditions.

Measurement and qualitative interpretation of echocardiograms will be performed primarily by technician-readers (Mary Paranicas, Lily Yee and Roseanne Morris) with extensive over-reading and supervision by physician-investigators (Drs. Richard Devereux and Mary Roman). Upon receipt of studies from the Field Centers, they will be logged by the data manager (Linda Gerber, Ph.D.) into a hard-copy book and into a computer that will already contain a master file with participant last names, initials, study numbers and other demographic information needed to assure unambiguous subjects identification and enable the quality-control steps to be described below. The preliminary reading form and unblinded log sheet (Appendices I and II) will be separated from the videotape. Videotapes will be assigned to technician and physician readers who will enter name, SHS number and date to assure a match into the Revue Center. After measurements are completed and transmitted electronically to the computer center, they will be merged electronically with demographic data to facilitate checking of echocardiographic measurements against ranges of expected values for body size and gender (Appendix IV and additional nomograms derived from previous population studies done at Cornell). Random samples of studies (five percent each) will be selected for duplicate readings to assess intra- and inter-observer variability. Studies with measurements or inter-reader differences that fall outside a priori limits for further verification as well as other quality control procedures, as well as the 5% sample of "in range" studies will be re-reviewed by the physician-readers. This mix of studies for re-review has been designed to minimize any potential systematic biases in the re-review process. Procedures for computerized data tracking and management will utilize standard data bases and ASCII files with limited custom programming done in house in coordination with the SHS Coordinating Center in Oklahoma. After studies have passed the quality-control verification steps, data will be transmitted to the Coordinating Center by electronic mail with mailed backup diskettes.

Videocassettes will be received weekly from each region or field center. After the studies are logged, they will be processed by technician-readers trained in the SHS reading protocol using a commercially available computerized system (Digisonics, Inc., Houston, Texas). Super VHS video-cassettes will be placed in the VCR and advanced to the start of each 2-D study (identified by code number). Parasternal long and short-axis 2-D views will be reviewed to ascertain correct M-mode beam angulation, and scored for semiquantitative wall motion of visualized wall segments. If the M-mode beam is correctly angulated, the technician-reader will choose the visually best LV cycles (up to 3), and identify the QRS onset for each cycle on the simultaneous ECG tracing to time end-diastolic measurements of interventricular septal and posterior wall thickness and LV internal dimension by the ASE (99) convention, and at the nadir of posterior septal motion for end-systole. For each set of measurements the depth (or Doppler velocity) and time calibrations will be repeated. They will measure these cycles (and record the videotape counter units of the chosen cycles to facilitate subsequent verification by a physician-investigator), and as an immediate quality-control step compare the measurements obtained by the CardioRevue Center to the videotaped images with the use of calipers as appropriate to obtain measurements by an independent method.

Evaluation of LV Structure: M-mode LV measurements will be made by the ASE (99) method (Figure 6). The ASE recommendations, in which measurements are made from leading edge to leading edge, time end-diastolic measurements at the QRS onset. If the M-mode beam is not correctly oriented, the 2-D parasternal long-axis recordings will be played backward and forward to find the cycle or up to three cycles that maximize(s) the LV cavity area. In this view, septal and PW thicknesses and LV internal dimension will be measured by the leading-edge technique (analogous to the ASE convention) at the level of the papillary muscle tips along an axis perpendicular to the LV walls (100). This procedure has been used for the past 6 years in the Cornell laboratory, and increases the proportion of subjects with measurable LVs by about 10%. LV mass values by this technique with the ASE correction (9) have proven nearly identical to those from good quality M-mode recordings in the same research subjects in the Cornell laboratory, indicating their interchangeability.

With the parasternal long-axis 2-D view on the monitor, the cycle illustrating the largest LV outflow tract and aortic root diameter will be visualized to measure the aortic anular diameter at the QRS onset between the hinging points of the two visualized aortic cusps, a measurement needed to calculate stroke volume (52). On this same 2-D image the aortic root diameter will be measured by the leading-edge technique at the level of the sinuses of Valsalva as described by Roman et al (72, 76), and the videotape will be advanced to end-systole (end of the T wave of the ECG) for measurement of left atrial diameter by the trailing edge to leading edge technique. The choice of the trailing edge of the posterior aortic wall, rather than the leading edge, is based on the fact that a space containing loose connective tissue exists between the aortic and left atrial walls that would otherwise be included in the left atrial diameter measurement.

The videotape will then be advanced to the apical 4- and 2-chamber views and played (several times if necessary) to allow completion by the reader of semi-quantitative scoring of wall motion (from normal to aneurysmal) on the 5-step scale recommended by the ASE (100). In addition, a summary impression of global LV systolic function (normal/abnormal/severely depressed) will be made. The videotape will be advanced to the 4-chamber view recording Doppler flow across the mitral anulus. Early and late diastolic flow will be traced by the leading edge (black-white interface) method to measure peak E and A velocities and the E and A time-velocity integrals on the three cycles illustrating the highest velocity. The videotape will then be advanced to the apical long-axis view illustrating transaortic flow and the aortic flow time-velocity interval measured on three cycles by the leading-edge black-white method as described by Dubin et al (52). The recordings of color Doppler flow will be used in conjunction with imaging information to record the presence, etiology and estimated severity of valvular regurgitation or stenosis by established methods (72, 74, 91).

Calculation of Derived Variables: After the technician reader has completed

accepting or correcting the initial primary measurements of cardiac dimensions, flow patterns and grading of the motion abnormalities, the data will be transferred to the Clinical Research Center computer, where mean values for these measurements will be utilized to calculate derived variables. A second step will merge blood pressure and body size measures for further calculations before range checks and additional physician-investigator verification of primary data.

M-mode measurements at end-diastole by ASE measurements are used to calculate LV mass by the anatomically validated formula (9):

$$\text{Left Ventricular Mass} = 0.8(1.04\,[(IVS + LVID + PWT)^3 - LVID^3]) + 0.6g$$

Estimates of LV mass by this method were closely related to actual LV weight at necropsy (r=0.90, p<0.001) in 52 adults.

Overall LV mass is the best measure of myocardial cell size, since the number of cardiac myocytes remains relatively constant after infancy, and is the most sensitive echocardiographic index of LV hypertrophy (46, 49). However, additional useful information is provided by the LV wall thickness/radius ratio, or "relative wall thickness" (RWT). This increases in proportion to chronic elevation of LV systolic pressure due to adaptive LV hypertrophy (101) and adds to LV mass for prediction of complications of hypertension (4). RWT is calculated from M-mode measurements as 2PWT/LVID (102); increased LV mass is classified as concentric hypertrophy if RWT is >0.41 and eccentric hypertrophy when RWT is normal (103). If LV relative wall thickness is increased but LV mass is normal, the subject is considered to have "concentric LV remodeling", an LV geometric pattern newly described from the Cornell Laboratory (4, 103).

Evaluation of Ventricular Performance and Load: Systolic function of a symmetrically contracting LV, such as occurs with uncomplicated hypertension, diabetes or alcoholism, can be assessed by measurement of the fractional shortening of LVID between end-diastole (d) and end-systole(s):

$$\text{Fractional Shortening }(\%) = [(LVIDd-LVIDs)/LVIDd] \times 100$$

If LV wall motion is uniform, fractional shortening is closely correlated with global LV ejection fraction, and is a simple substitute for it (104).

Because ejection-phase indices of LV performance are highly dependent on afterload, measurement of myocardial afterload is helpful in determining whether or not observed ventricular function reflects normal myocardial contractility. The most direct measure of myocardial afterload is end-systolic stress (ESS), which can be measured using end-systolic LV measurements by the ASE convention and cuff blood pressure, measured with the subject on the examining table at the end of the echocardiogram, in a catheterization-

validated formula (105):

$$ESS = (0.334 \text{ x SBP x LVIDs})/[PWTs \text{ x } (1 + PWTs/LVIDs)]$$

A close inverse relation exists between fractional shortening and ESS in both normal and hypertensive subjects (59, 69, 106-107), which becomes most linear when ESS is plotted on a logarithmic scale (ESS_{10}). Expression of observed fractional shortening as a percent of that predicted fro end-systolic stress provides an afterload-independent measure of LV contractile performance. Afterload-corrected endocardial fractional shortening is subnormal in patients with congestive cardiomyopathy (51) and normal or elevated in patients with uncomplicated essential hypertension (59, 61). Recent research from the Cornell laboratory suggests that fractional shortening calculated at the left ventricular midwall is more appropriately matched to the mean level of end-systolic stress across the ventricular wall than is conventional endocardial fractional shortening for use in stress-shortening relations (108). This approach will also be explored.

2-D Evaluation of LV Performance will rely primarily on evaluation of LV function by the semi-quantitative scoring system recommended by the ASE (100). This system utilizes parasternal short-axis views at mitral valve and midventricular level, apical 2 and 4-chamber views, and parasternal and apical long-axis views to visualize 6 wall segments in each short-axis plane and 4 segments in the LV apical region. Careful adherence to the described protocol permits scoring of wall motion in all segments in more than 80% of subjects studied under difficult circumstances (portably in a CCU setting) and should permit at least this high a yield in SHS participants.

1.4.8 Feedback to Field Centers and Quality Control

The preliminary reports prepared by the Field Center technicians will be reviewed and comments made confirming the quality of recordings and measurements or indicating needed corrections and how to accomplish them. These comments will be returned weekly to the Field Center technicians. After the technician-reader has checked the computer print-out of primary and derived measurements for appropriate correspondence to the primary recordings from which they are derived, blinded studies will be arranged in batches for verification by a physician investigator. The M-mode stripchart recordings and print-out of measurements on all studies on a videotape will be presented in sequence.

Because of the central importance of LV mass and systolic function, LV measurements and grading of systolic LV function will be reviewed and verified or corrected by the investigators after the initial set of verified measurements have been merged by the data manager with demographic data (age, gender, height, weight and arterial pressure) to permit data-based quality control. The latter steps will include: 1) blinded re-reading by the same technician-physician pair of 5% of studies using half of the duplicate M-mode LV stripchart recording made at the field centers) to assess inter-reading

variability; 2) blinded reading of the duplicate M-mode recording and videotape of another 5% of studies by a different technician-physician pair (to assess inter-reader variability) and 3) re-review of all studies with measurements that fall outside of the normal to mildly abnormal range (based on the fact that erroneous extreme values will have the greatest impact in obscuring or distorting true biological relationships). Additional measurement sets that result from these duplicate readings will be entered into the computer under separate codes; the final physician-investigator verified data on each subject will constitute the primary data set transmitted by electronic mail with diskette backup to the Coordinating Center. To maximize feedback to technician-readers, the investigators will conduct part of their verification of measurements and the above quality-control steps, as well as final adjudication of discrepant measurements, in regular review sessions with the technician-readers.

1.4.9 Reports and Alerts

After measurements and interpretations are finalized by the Reading Center, a report including the average value of measurements on multiple cycles and clinical interpretation of the normality/abnormality of the study will be generated by the Cardiorevue Center. Copies of this will be a) returned to the Field Centers for inclusion in the subjects' IHS charts and b) sent to Dr. Oopik for the Dakotas, Dr. Ali for Oklahoma and a physician to be identified in Arizona who will determine what additional clinical feedback and follow-up should be initiated. For a limited number or potentially life-threatening tamponade or intra-cardiac masses) the Field Center sonographers will contact the physician to initiate an alert. Videotapes documenting these findings and others that do not trigger urgent alerts but are considered alarming by the sonographer will be sent to the Reading Center the day they are detected.

1.4.10 Wrap-Up/Data Analysis

Activities of the Echocardiography Reading Center from November 1995 to June 1996 will be devoted to final data processing and quality control and to working with the Coordinating Center for data analysis. It is planned to retain 50% effort of technician readers for the first two months of this period to assure careful processing of studies from the end of the performance period. During this period, the physician-investigators will complete verification of all echocardiographic data, and will oversee with the data manager a final step in quality control that will necessitate interaction with the Coordinating Center. Subgroups of study participants will be defined on non-echocardiographic criteria as being normal or having specific conditions (e.g., previous myocardial infarction, extreme obesity) and the distribution of measurements and qualitative/semiquantitative scoring of LV wall motion examined to determine whether they are appropriate for the group in question. Of the 4,050 subjects expected to be studied in exam II, it is anticipated that the planned strategy of echocardiographic performance and measurement will yield LV measurements and semiquantitative assessment of LV function in about 80% or 3,240,

with qualitative grading of LV function available in over 90% of SHS participants.

Once the echocardiography data set is finalized, the investigators and data manager of the Echocardiography Reading Center, in cooperation with the Coordinating Center at the University of Oklahoma, will test the following hypotheses. These hypotheses will be tested in the entire population, in subsets thereof, and in study participants who are free of prevalent myocardial infarction or clinically or echocardiographically defined valvular heart disease.

1) The distribution of measures of LV size, mass and function in SHS participants differs from that in Caucasian populations. This hypothesis is based on the high prevalence in SHS participants of obesity and diabetes, known stimuli to LV enlargement, hypertrophy and dysfunction (55, 109). Comparison groups will include a) Caucasian subjects from employed population samples studied in New York (54, 87) with proportionate sampling of hypertensive subjects to correct for their over-representation in our reported studies and b) the Framingham general population sample, which is overwhelmingly Caucasian and which was studied by echocardiographic methods that were carefully coordinated with joint echocardiogram reading by Dr. Devereux and Daniel Savage to assure comparability of measurements to those at Cornell (4, 69, 110-111). Variables to be considered are LV septal, posterior and relative wall thickness, internal dimension and mass; and LV fractional shortening. Statistical testing of between-groups differences will use Student's t-test (or Welch's approximate t for unequal variances) for normally distributed data or the Mann-Whitney test if data are not normally distributed. Because of reported differences in prevalence of diabetes and other risk factors among native Americans in different regions (Arizona, Oklahoma, South Dakota) surveyed in the SHS, findings in subjects in the three regions will be compared by one-way analysis of variance followed (if significant differences are revealed) by the Scheffe test. If homogeneity is found among SHS regions the pooled data will be compared with that from Caucasian groups; otherwise, comparison will be made using both pooled and region-specific SHS data.

2) Echocardiographic LV mass and dysfunction are strongly associated with prevalent clinical heart disease and subsequent mortality in SHS participants: This prediction is based on observed relations of LV findings to prevalent coronary, valvular and myocardial heart disease in other populations (21, 51, 69) and on the strong, independent predictive value of baseline echocardiographic LV mass for morbid and mortal events in the Framingham general population sample (3, 13), in hypertensive patients in long-term follow-up at Cornell (4, 11) and in patients undergoing coronary arteriography (15). The cohort will be divided based on clinical examination, ECG, and medical/hospitalization record data into groups a) with specific classes of disease; b) considered to be cardiovascularly normal, or c) fall in-between due to symptoms or medication use without a specific cardiac diagnosis. Echocardiographic variables will be compared among groups by ANOVA followed by the Scheffe test. In addition to testing

the applicability of previous findings to Native Americans, appropriate classification of subjects will permit comparison of the cardiac effects of preclinical conditions (diabetes, extreme obesity or alcohol excess) to those of established heart disease (e.g., myocardial infarction). To add a longitudinal aspect to this facet of SHS, follow-up for mortality can be done at relatively low cost through the National Death Index and follow-back studies beyond the duration of the present funding period; ascertainment of non-fatal morbid events (myocardial infarction, etc.) will only be feasible over the long-term if the SHS is continued beyond the proposed period.

3) Increased body fat mass is a strong correlate of LV enlargement and hypertrophy independent of effects of lean body mass, blood pressure or gender. This prediction is based on studies that have used simple ratios of body proportions (body mass index) or indirect estimation from skinfold thicknesses (18, 21, 55). In SHS, percent body fat will be calculated by bioelectric impedance, a more direct albeit still approximate method, and used with body weight to estimate adipose and non-adipose body mass. The impact of adipose body mass and its distribution on LV dimensions and functional measurements will be tested by: a) comparing LV measurements in strata of SHS men and women with normal or elevated adipose body mass by appropriate parametric or non-parametric methods, b) evaluating the independent relations of adipose and non-adipose body mass as well as blood pressure, gender and physical activity to LV mass and other LV dimensions, and c) comparing LV mass between groups of subjects in whom increased adipose mass occurs in either a gyroid or android distribution. The strength of adipose body mass as a stimulus to increased LV mass will be estimated by examining the relations of LV mass to height in normal weight and strata of increasingly overweight SHS participants. Application of this approach in a joint study at Cornell and the Universities of Naples and Cincinnati (58) has already shown that LV mass exhibited a curvilinear relation (between a second-power and a third-power one) to height in 611 normal-weight subjects; findings in 56 overweight subjects suggested that a kg of adipose body mass is associated with about half as much LV mass as a kilogram of lean body mass. The SHS cohort will provide an ideal setting in which to define more precisely these relationships, because of its population base, relative ethnic homogeneity, high prevalence of obesity and ability to estimate body fat mass and its distribution. Mr. Tarquin Collis, a Cornell University Medical College student, will perform initial analyses of this question on the first 800 subjects with LV measurements during a student research fellowship covering the 1993-1994 academic year.

4) Impaired glucose tolerance and overt diabetes are associated with impaired LV systolic performance and abnormal diastolic filling, independent of effects of other variables: This prediction is based on an extensive clinical, pathological, and experimental literature linking both naturally-occurring and induced forms of diabetes to evidence of cardiac dysfunction or frank cardiomyopathy. Analyses will consider glucose metabolism both as a categorical variables (normal, impaired, overt diabetes) and as a continuous variable (fasting and 2-hour post glucose-load levels of plasma glucose). Dependent variables will be measures of LV systolic performance (M-mode fractional shortening and

fractional shortening as a percent of that predicted for ESS, and 2-D semi-quantitative LV wall motion score and qualitative scoring of global LV systolic function) and of LV diastolic filling (Doppler E and A velocities and integrals and their ratios). Because of evidence from Framingham that diabetic subjects may have increased LV wall thicknesses, which may cause an overstatement of LV systolic performance when fractional shortening measured at the endocardium is related to the mean level of end-systolic stress across the LV wall (108), midwall LV fractional shortening will also be calculated. Groups categorized by normality of glucose metabolism will be compared with regard to these variables by analysis of variance followed by the Scheffe test, while relations between measures of glucose metabolism and LV function will be assessed by univariate and multivariate regression techniques, as previously described. Because of potentially important confounding effects of obesity and age, analyses will be repeated in strata of normal or increased relative body weight with adjustment or additional stratification for age.

5 and 6) Alcohol intake and fasting insulin level are positively related to LV mass, independent of body habitus or the level of resting, alcohol-free blood pressure: These predictions are based on the finding of a positive alcohol-LV mass relation in the overwhelmingly Caucasian Framingham population (44), and the recent observation of a positive relation between insulin and LV mass by Phillips et al. Primary analyses will utilize alcohol intake, insulin level and LV mass as continuous variables both in the entire population and in strata with defined ranges of relative body weight, normality of glucose metabolism, etc. Relations between variables will utilize linear (Pearson's) correlation between primary pairs of variables and multiple linear regression analyses, to determine the independence of observed relations from effects of potential confounding variables (blood pressure, gender, relative body weight, etc.). If, as expected, primary data are not normally distributed, statistically significant results of linear regression analyses will only be accepted if they are confirmed by non-parametric methods (e.g., Spearman's rank-order test).

7) Valvular heart diseases are more prevalent than in Caucasian populations, and will be related to a history of rheumatic fever and with obesity: These predictions are based on a) historic evidence of rheumatic fever outbreaks on some Indian reservations at a time when its incidence had fallen to low levels among White Americans, and b) evidence obtained at Cornell that significant valvular regurgitation due to mitral valve prolapse or idiopathic aortic root dilatation was associated with increased BMI (72, 80).

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APPENDIX 1
THE STRONG HEART STUDY II

Echocardiogram Log

Center:		Tape:		Log for SHS Echo Tape Box		
Counter	Last Name	Initials	SHS #	Soc Sec #	Date	Sonographer #

APPENDIX 2

THE STRONG HEART STUDY II

Unblinded Log for Echocardiogram

Center:		Tape:		Unblinded Log for SHS Echo Tapes								
Counter	Name (Last/First)	SHS#	SS#	SBP	DBP	SEX	DOB	Ht.	Wt.	Date	Sonographer	

APPENDIX 3

THE STRONG HEART STUDY II

PRELIMINARY ECHOCARDIOGRAM INTERPRETATION

DATE: _____   SHS#: _____

CENTER: _____   IHS#: _____

TAPE: _____   Subject's Last Name: _____

COUNTER: _____   Subject's Initials: _____

DIMENSIONS (cm)   Image Orientation   Interface

	m	2-D		
IVSd	_	_	G _ F _ P _ NG _	G _ F _ P _ NG _
LVIDd	_	_	G _ F _ P _ NG _	G _ F _ P _ NG _
PWTd	_	_	G _ F _ P _ NG _	G _ F _ P _ NG _
LVIDs	_	_	G _ F _ P _ NG _	G _ F _ P _ NG _
LA	_	_	G _ F _ P _ NG _	G _ F _ P _ NG _
Ao	_	_	G _ F _ P _ NG _	G _ F _ P _ NG _

2-D WALL MOTION   Image Orientation   Interface

Normal _____ Borderline _____   G _ F _ P _ NG _   G _ F _ P _ NG _
Global _____ Segmental Abnl _____   G _ F _ P _ NG _   G _ F _ P _ NG _

DOPPLER   Beam Orientation   EnveloPeDefinition

Peak Ao _____   G _ F _ P _ NG _   G _ F _ P _ NG _
Peak EVOT _____   G _ F _ P _ NG _   G _ F _ P _ NG _
Mitral E _____   G _ F _ P _ NG _   G _ F _ P _ NG _
Mitral A _____   G _ F _ P _ NG _   G _ F _ P _ NG _
MR: None_ 1+ 2+ 3+_ 4+_   G _ F _ P _ NG _   G _ F _ P _ NG _
AR: None_ 1+_ 2+_ 3+_ 4+_   G _ F _ P _ NG _   G _ F _ P _ NG _

Mitral Valve:	Normal____	Abnormal____	**Clinical Alerts:** Yes__ No__
Aorta / LA:	Normal____	Abnormal____	**Severe AS** _____
Left Ventricle:	Normal____	Abnormal____	**Intracardiac Mass** _____
Doppler:	Normal____	Abnormal____	**Cardiac Tamponade** _____

Study performed by: _____

APPENDIX 4
THE STRONG HEART STUDY II

THE NEW YORY HOSPITAL CORNELL MEDICAL CENTER
ECHOCARDIOGRAPHY REPORT

Patient Name:	SAMPLE	**SHS #:**	111-11-11
Center:	Cornell Med Center	**IHS #:**	222-22-22
DOB:	5/5/36	**Date:**	6/29/93
Height:	72 in Weight: 230 lb	**BSA:**	2.26
BP:	115/51 Sex: M	**Tape:**	001

M - M O D E

	NORMALS		NORMALS
Septum		**LV**	
Dias Thick	10.2 mm(6.0-11.0)	Dias Dim	8.51 cm(3.70-5.60)*
Sys Thick	14.4 mm	Sys Dim	6.17 cm
Thick Frac	0.42 (0.30-0.64)	Short Frac	0.30 (0.28-0.44)
Dias Thick	10.6 mm(6.0-11.0)	Dias Vol	396 ml
Sys Thick	17.4 mm	Sys Vol	175 ml
Thick Frac	0.64 (0.30 - 0.64)*	Stroke Vol	221 ml
Dias Thick	11.0 mm (6.0 11.0)*	Eject Frac	56 %
Sys Thick	15.2 mm	Dias Dim	8.68 cm (3.70-5.60)*
Thick Frac	0.38 (0.30-0.64)	Sys Dim	5.89 cm
		Short Frac	0.32 (0.28-0.44)
LVWP		Dias Vol	414 ml
Dias Thick	11.0 mm (6.0-11.0)*	Sys Vol	172 ml
Sys Thick	15.2 mm	Stroke Vol	241 ml
Thick Frac	0.38 (0.30-0.64)	Eject Frac	58 %
Dias Frac	12.7 mm (6.0-11.0)*	Dias Dim	8.60 cm (3.70-5.60)*
Sys Thick	16.1 mm	Sys Dim	5.97 cm
Thick Frac	0.27 (0.30-0.64)*	Short Frac	0.31 (0.28-0.44)
Dias Thick	13.6 mm (6.0-11.0)*	Dias Vol	405 ml
Sys Thick	13.6 mm	Sys Vol	178 ml
Thick Frac	-0.00 (0.30-0.64)*	Stroke Vol	227 ml
		Eject Frac	56 %
MV			
Excursion	11 mm (22-30)*	**Aorta**	
EF Slope	130 mm/s (70-150)	Root Diam	4.70 cm (2.00-3.70)*
EPSS	38 mm	Root Diam	4.62 cm (2.00-3.70)*
Excursion	14 mm (22-30)*	Root Diam	4.79 cm (2.00-3.70)*
EF Slope	153 mm/s (70-150)*		
SPSS	40 mm	**LA**	
Excursion	10 mm (22-30)*	Dimension	3.69 cm (1.90-4.00)
EF Slope	111 mm/s (70-150)	Dimension	3.81 cm (1.90-4.00)
EPSS	38 mm		
		AV	
		Cusp Sep	2.6 cm (1.5-2.6)*
		Cusp Sep	2.8 cm (1.5-2.6)*
		Cusp Sep	2.8 cm (1.5-2.6)*

2 - D

Aorta - PLA
Annulus	4.0 cm
Root Diam	4.0 cm
Annulus	3.5 cm
Root Diam	3.5 cm
Annulus	3.8 cm
Root Diam	3.5 cm

LVPW - PLA
Dias Thick	1.3 cm
Sys Thick	1.3 cm
Dias Thick	1.2 cm
Sys Thick	1.0 cm
Dias Thick	1.4 cm
Sys Thick	1.3 cm

Septum - PLA
Dias Thick	1.9 cm
Sys Thick	2.0 cm
Dias Thick	2.0 cm
Sys Thick	1.8 cm
Dias Thick	1.9 cm
Sys Thick	1.9 cm

LA - PLA
Sys Diam	4.8 cm
Sys Diam	4.9 cm
Sys Diam	4.7 cm

LV - PLA
Dias Dim	5.9 cm(3.5-6.0)
Sys Dim	3.6 cm(2.1-4.0)
Shrt Frac	0.39 cm
Wall Thck	1.6 cm
M Wall Str	56.70
Dias Dim	5.9 cm (3.5-6.0)
Sys Dim	4.2 cm (2.1-4.0)*
Shrt Frac	0.29
Wall Thck	1.6 cm
M Wall Str	84.25
Dias Dim	5.7 cm (3.5-6.0)
Sys Dim	4.1 cm
Shrt Frac	0.28
Wall Thck	1.6 cm
M Wall Str	68.87

LV - Short Axis
Mn Diam-D	6.8 cm
Mn Diam-S	4.8 cm
Mn SF	31 %
Mn Wall Th	1.3 cm
Mn R/Th	2.53
Mn Diam-D	6.5 cm
Mn Diam-S	4.3 cm
Mn SF	33 %
Mn Wall Th	1.3 cm
Mn R/Th	2.47
Mn Diam-D	6.7 cm
Mn Diam-S	4.8 cm
Mn SF	28 %
Mn Wall Th	1.4 cm
Mn R/Th	2.46

DOPPLER

AV
Time	346 msec
TVI	43.6 cm
Peak vel	184.1 cm/s
Mean vel	129.0 cm/s
Peak Grad	14 mmHg
Mean Grad	7 mmHg
AC/ET	0.30
Pk Acc Rate	4978 c/s/s
Time	360 msec
TVI	45.0 cm

MV
TVI	19.0 cm
Time	630 msec
Pres Hf Tm	36 msec
Area	6.16 sq cm
E	72.47 cm/s
A	37.03 cm/s
E/A	1.22
Acc Time	83 msec
1st 1/2 FF	0.65
1st 1/3 FF	0.43

Name SAMPLE   Pat #: 111-11-11   Date: 6/28/93

AV
   Peak vel   185.1 cm/s
   Mean vel   127.8 cm/s
   Peak Grad   14 mmHg
   Mean Grad   7 mmHg
   AC/ET   0.37
   Pk Acc Rate   1833 c/s/s
   Time   329 msec
   TVI   44.6 cm
   Peak vel   189.0 cm/s
   Mean vel   131.9 cm/s
   Peak Grad   14 mmHg
   Mean Grad   8 mmHg
   AC/ET   0.45
   Pk Acc Rate   10464 c/s/s

MV
   Dec Time(av)   124 msec
   Atrial FF   0.42
   TVI   12.0 cm
   Time   298 msec
   Pres Hf Tm   76 msec
   Area   2.90 sq cm
   E   74.27 cm/s
   A   73.34 cm/s
   E/A   1.01
   Acc Time   82 msec
   1st 1/2 FF   0.79
   1st 1/3 FF   0.53
   Dec Time   259 msec
   Atrial FF   -0.11
   TVI   19.6 cm
   Time   787 msec
   Pres Hf Tm   73 msec
   Area   3.02 sq cm
   E   72.07 cm/s
   A   58.33 cm/s
   E/A   1.24
   Acc Time   83 msec
   1st 1/2 FF   0.66
   1st 1/3 FF   0.49
   Dec Time   248 msed
   Atrial FF   0.42

COMMENTS

M-MODE STUDY:

   Dilated left ventricle

2-D STUDY:

   Enlarged aortic root
   Diastolic AMVL fluttering suggestive of AI
   Normal left atrial size
   Left ventricular enlargement, seve!e
   Eccentric left ventricular hypertrophy

DOPPLER:

   Severe aortic insufficiency
   Mild mitral regurgitation

Parasternal Short Axis-Basal Level   Parasternal Short Axis-Mid Level
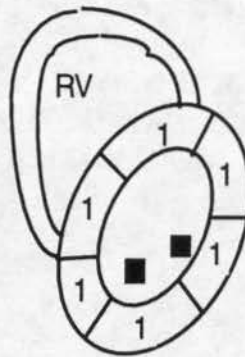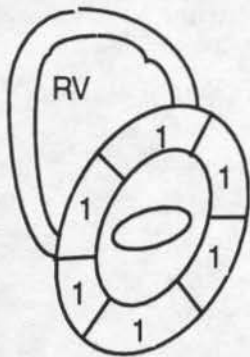
Parasternal Short Axis-Apical Level
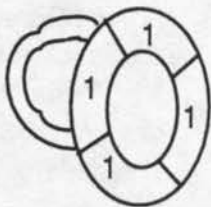
QUALITATIVE WALL MOTION TABLE

	BASAL	MID	APICAL
ANT. SEPTUM	Normal	Normal	Normal
ANTERIOR	Normal	Normal	Normal
ANT. LAT.	Normal	Normal	Normal
INF. LAT.	Normal	Normal	Normal
INFERIOR	Normal	Normal	Normal
INF. SEPTUM	Normal	Normal	Normal

Wall Motion Score Index : 1.0

APPENDIX 5
THE STRONG HEART STUDY II

Sample of Results Report for the Echocardiographic Findings

THE NEW YORK HOSPITAL - CORNELL MEDICAL CENTER
ARIZONA / DAKOTAS / OKLAHOMA
ECHOCARDIOGRAPHY REPORT

Date	01/05/1994		Name	xxx
Study Date	09/22/1993		SHS #	xxxxxx
Reading Date	10/05/1993		Age	68
Tape Number	C10-93		Sex	F
Counter	37:25 - 47:38		BSA	1.91 m2
Heart Rate	75 bpm		Ht	160 cm
Blood Pressure	140/66 mmHg		Wt	88 kg

DIMENSIONS (cm)

	Actual	Normal
Septum (d)	.90	<=1.1
Septum (s)	.90	...
LV Wall (d)	.80	<=1.1
LV Wall (s)	1.10	...
LA	3.00	<=3.8
Aorta	3.30	<=3.6
LVID (d)	6.80	<=5.4
LVID (s)	5.70	<=3.9

DERIVED VARIABLES

	Actual	Normal
LVED Volume	125.34	50-90 ml/m2
LVES Volume	83.85	15-35 ml/m2
Stroke Index	41.49	32-58 ml/m2
Ejection Fraction	.33	.55-.75
Cardiac Output	3.11	liters/min
LV Mass Index	130.94	110 g/m2
RWTd	.24	<.45
Fractional shortening	16.18	>=26%

DOPPLER VELOCITIES

	Actual	Normal
LVOT	1.25	<=1.50 m/s
Mitral 'E'	.77	<=0.90 m/s
Mitral 'A'	.97	<=0.60 m/s

DIAGNOSTIC COMMENTS:

1. Dilated left ventricle
2. Eccentric left ventricular hypertrophy
3. Moderately decreased estimated ejection fraction
4. Left ventricular enlargement, moderate
5. Anterior wall hypokinesia, mild
6. Interventricular septal akinesia
7. Anterior lateral wall hypokinesia, mild

8. Apical hypokinesia, severe
9. Thickened mitral annulus
10. Doppler color flow mapping reveals mild (1+) mitral regurgitation.
11. Normal mitral valve flow pattern
12. Normal mitral valve with no evidence of rheumatic heart disease, IHSS or mitral valve prolapse.
13. Normal aortic root, valve and left atrial size and motion.
14. Sector scan confirms the above. The aortic valve is trileaflet. The right ventricle and atrium are normal.
15. Abnormal echo exam consistent with ischemic cardiomyopathy
16. MARY PARANICAS, B.A.
17. RICHARD B. DEVEREUX, M.D.

By _____

2. SPIROMETRY - MANUAL OF OPERATIONS

2.1   Forward

This manual serves three purposes:

•   a study guide for training of technicians to perform pulmonary function testing

•   a practical "how-to" reference guide to be used by clinic staff during the study

•   documentation of the pulmonary function testing procedures for analyses and manuscript preparation.

2.2   Background

Rationale for Pulmonary Function Testing

1.   According to the results of the Framingham study and many others, the Forced Vital Capacity (FVC) from the spirometry test is an excellent independent predictor of mortality from cardiovascular diseases, even after adjusting for cigarette smoking (1-2). It is hypothesized that this "lung test" predicts heart disease mortality because the FVC decreases when lung congestion occurs due to early (preclinical) left-sided heart failure (CHF). Addition of spirometry testing in the Strong Heart Study should, therefore, allow better prediction of CVD morbidity and mortality.

2.   Measurement of the slow vital capacity (SVC) in the standing position will also be performed in those unable to perform high quality FVC maneuvers and those with a low FVC. This addition will allow us to better differentiate between the various causes of a reduced FVC. The FVC, but not the SVC, is reduced in moderate to severe airways obstruction (due to asthma or COPD). We estimate that about 15% of the participants will have some degree of airways obstruction. The high intra-thoracic pressures generated during the forced FVC maneuver in these participants makes their narrowed airways close completely towards the end of the maneuver, trapping air in the alveoli, causing an under-estimation of the true vital capacity (as measured by the SVC). The measurement of a normal SVC in these participants will rule out the presence of a superimposed restrictive process (such as pneumonia, congestive heart failure, or obesity).

The SVC breathing maneuver is much easier to perform than the FVC maneuver. This is important in the 6-10% of participants who are not highly motivated to perform the athletic-type breathing maneuvers required by the FVC test.

3.   The FEV1 from the spirometry test is the best predictor of morbidity and mortality from

chronic obstructive pulmonary disease (COPD) due to cigarette smoking (3-4). COPD is one of the ten leading causes of morbidity and mortality in American Indians. Portions of an American Thoracic Society standardized respiratory questionnaire (5) regarding asthma, chronic cough, sputum production, and smoking cessation attempts have been added to the study questionnaires. These will allow the assessment of associations between cigarette smoking, respiratory symptoms, and respiratory diagnoses and pulmonary function results (and the later development of overt respiratory disease, if follow-up studies are later performed).

4.   When the FEV1 results show airways obstruction in a current smoker, that individual has been demonstrated to be susceptible to the pathologic effects of cigarette smoking and is at very high risk for disability and death from COPD (6) or lung cancer. Participants who are smokers and have abnormal spirometry results will, therefore, be referred to a smoking cessation program (not funded by this study).

5.   Predicted pulmonary function values for American Indians are not well established. In one study of nonsmoking, adult Navajo Indians without lung disease, spirometric values were found to be statistically different (but close) to larger studies of normal whites (7). The values of Navajo Indians, however, may be quite different from other Indian tribes. The normal population studies used by most PF laboratories did not test any Native American subjects (8). Rhoades states that "There is a great need for additional study of ventilatory function in American Indians, including the establishment of normal values" (9). Addition of PF testing to the Strong Heart Study will accomplish this, which in turn will allow more sensitive and specific diagnostic testing of Native Americans for asthma, COPD, and restrictive lung diseases.

6.   American Indian mortality and hospitalization rates during the 1980s due to respiratory diseases were recently reviewed (9-10). The most prominent (in order of mortality rate) are pneumonia, lung cancer, COPD, and tuberculosis. Rates vary considerably between geographic areas of the country: Aberdeen, Billings, and Bemidji rates are higher, associated with higher cigarette smoking rates (11). Addition of the standardized respiratory questionnaire will help to quantitate the prevalence of these diseases in the Strong Heart Study participants when they have been diagnosed by a physician. Pulmonary function tests will add objective data concerning the prevalence rates of obstructive lung disease (COPD and asthma) in their early (preclinical) stages, and lung infections which leave permanent scarring resulting in an abnormally low vital capacity (FVC). (Spirometry, however, is NOT helpful in the detection of lung cancer.)

7.   The addition of PF testing is an opportunity to bring more attention to other preventable respiratory diseases in this population. The death rate from TB in American Indians has declined dramatically during the last 30 years, but still appears high in Alaska and North Dakota, relative to whites (9). Lobectomies or other lung surgery for TB will frequently reduce the vital capacity, so this effect could be studied by spirometry.

Coccidioidomycosis (cocci, valley fever) is endemic in Southwestern Indians who live in or visit the Sonoran desert (including Arizona) and may affect pulmonary function when disseminated. Questions regarding tuberculosis and cocci exposure, and history of previous diagnosis and treatment (as currently done only at the Dakota clinic) will be added to the respiratory questionnaire.

Tuberculosis skin tests will be applied when appropriate (as specified by American Thoracic Society guidelines), and Cocci skin tests will be applied to participants in Arizona. The participant will return in 2-3 days to have the test reaction measured by community health workers. Those with positive results will be referred for appropriate treatment.

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2.3 Definitions

A/D CONVERTER is a small electronic interface card mounted inside the spirometer which changes the analog voltages from the spirometer potentiometer and temperature sensor to digital numbers that the computer can understand. These are transferred to the personal computer via the RS-232 serial interface.

ARCHIVAL FLOPPY DISK is the floppy disk which stores a backup copy of participant test results, to be stored at the Field Center in case the PF Workstation's hard disk crashes or the Mailer floppy disk is misplaced by the U.S. Postal Service.

ATPS is the condition of air inside the spirometer - Ambient Temperature and Pressure, and Saturated with water vapor. The ambient temperature of the spirometer is usually lower than body temperature; this has the effect of cooling and contracting the volume of air exhaled into the spirometer.

ATS is short for American Thoracic Society, the scientific branch of the American Lung Association - the Easter Seal folks. The ATS promotes accurate spirometers by recommending spirometry standards.

BACK EXTRAPOLATION is the standard method used to determine "time zero" when measuring the FEV1. The amount of slowly exhaled volume at the start of the maneuver excluded from the FEV1 by this technique is called the back extrapolated volume (BEV or EV). The BEV should be less than 5% of the vital capacity, otherwise the maneuver is considered to have started too slowly.

BTPS stands for Body Temperature (usually 37 degC) and Pressure, and Saturated with water vapor (100% humidity), which is the condition of air inside the lungs before it is exhaled into a spirometer. ATS standards require that volumes and flows be reported as if they were under these conditions.

CALIBRATION SYRINGE is a large metal cylinder with a rubber sealed piston used to check the volume accuracy of spirometers. The ATS recommends that it be 3.00 liters in size and we use a sturdy aluminum model made by Hans Rudolph.

COPD stands for Chronic Obstructive Pulmonary Disease, a general term for lung disease

caused by cigarette smoking - a mixture of emphysema, bronchitis, and hyperreactive airways.

EV (see Back Extrapolation)

FET is short for Forced Exhalation Time. The FET should be at least ten seconds for the FVC maneuver to be considered acceptable, otherwise the FVC may be underestimated. The FET is displayed on the incentive screen as the Duration.

FEV1 is the most important spirometry variable, short for Forced Expiratory Volume in one second. It is convenient to think of it as the average flow rate during the first second of the FVC maneuver. It is reduced with airflow obstruction.

FEV1/FVC RATIO is the most sensitive and specific index of airways obstruction measured by a spirometer. It is normally above 70%.

FLOPPY DISKS are removable, rather slow, computer storage media. The personal computer's floppy disk (drive A:) uses high density (HD) 3 1/2 inch floppy disks which can each store up to 1.44 million characters (Mbytes).

FLOW-VOLUME CURVE is the graph obtained from a forced exhalation maneuver plotted with flow on the vertical axis and volume on the horizontal axis. When compared with the traditional spirogram, it has the advantage of allowing easy recognition of unacceptable or poorly reproducible maneuvers and disease patterns.

FVC is the Forced Vital Capacity, the volume of air exhaled during the maneuver named after it. The subject takes as deep a breath as possible and then quickly exhales as much air as possible. The FVC is reduced with restrictive disorders.

HARD DISK is the personal computer's fast, mass storage device (drive C:) which stores millions of characters.

OBSTRUCTION is a decrease in maximal airflow rates caused by airway narrowing. The FEV1/FVC ratio and the FEV1 are both decreased.

PEF stands for Peak Expiratory Flow Rate, the highest flow measured during the FVC maneuver. It is a good index of effort used at the onset of the maneuver. It can be seen on a flow-volume curve but not on a traditional volume-time spirogram. Inexpensive $10 hand-held instruments can also measure PEF with better than 10% accuracy. These peak flow meters will be used to assess the lability of airways obstruction in a subset of the CHS population.

2.4   Methods Summary

Daily Procedures

Calibrate Instruments
- Power-up computer and spirometer
- Run leak and volume checks
- Wash your hands

Identify the participant
- Enter name, ID number, age, height, weight

Perform FVC maneuvers
- Demonstrate the FVC maneuver
- Obtain 3 acceptable FVC maneuvers
- Review maneuver quality
- Measure Slow VC if unable to perform FVCs
- Add comments
- Print and store the results

Clean Equipment at the end of the day
- Clean breathing hoses
- Rinse and dry hoses overnight

Weekly Procedures

Monday mornings
- Run leak and volume cal checks
- Perform a biologic control test

Friday afternoons
- Remove spirometer shell
- Clean internal compartment
- Rinse and dry overnight

2.5   Description of the PF Workstation

A dry-sealed volume spirometer is connected to a personal computer using a 12 bit analog to digital (A/D) interface. The spirometer is equipped with a potentiometer (pot) which changes the mechanical motion of the spirometer bell into a voltage which is proportional to exhaled volume. An electronic sensor measures the spirometer temperature for automated BTPS corrections. The A/D converter, mounted on a board inside the spirometer takes the analog voltages, converts them into digital numbers and sends them to the computer via an RS-232 serial interface. The computer

then calculates the exhalation time (FET) and airflow rates (FEV1) using a crystal controlled clock and stores all the results in RAM memory. The results are stored on the hard disk, printed, and copied to a diskette to be mailed to the PF Reading Center.

2.6   Main Menu

The MAIN MENU is automatically displayed when the computer's power is turned ON. If you are faced with the DOS prompt C:> type **GO** The MAIN MENU is the control center or hub of the system. Moving from one function to another is performed by going back to the MAIN MENU first.

You usually move forward within a program by pressing either the Enter key or the spacebar. Directions are often given at the bottom of the screen. If you obtain a program or screen by mistake, you can usually get back to the MAIN MENU by pressing the Esc key.

Select the desired program from the MAIN MENU by highlighting your selection using the cursor (arrow) keys. Then press the Enter key. An alternate method for experienced users is to merely press the three letter code for the program (not followed by Enter).

The first column of selections, under the heading **PRE:Tests** lists the most frequently used programs in the order in which they are usually selected:

INF - Enter patient information Used to enter the name, ID number, age, height, etc for a new participant.The name of the "current participant" is given in parentheses on this line.

FVC - Forced Vital Capacity Guides performance of FVC maneuvers. Flow-volume curves are displayed on the screen for quality control.

EOS - End the Test Session Asks you for comments, then prints a report for the participant and his/her physician and a tabular report for the participant's on-site CHS chart. The data are then stored in a directory on the hard disk.

SVC - Slow Vital Capacity If the participant can't perform good FVC maneuvers, the slow VC test should be done. It requires very little effort.

TXT - Enter Comments You may go back and edit your comments about what happened during testing at any time.

2.7   Participant Information

Select "INF - Identify the Participant for the MAIN MENU." If you did not complete a leak and volume cal check today, you will be instructed to do so at this time, before testing a participant (see the CALIBRATION section of this manual).

Enter or verify the information requested in each box. End each entry by pressing the ENTER key. Every item must be entered in order to calculate predicted values.

Name: Enter the participant's last name, a comma, then his first name (up to a maximum of 22 letters) Use all capital letters. *Don't add a space after the comma.* Press F2 here to edit the currently selected participant's data (instead of entering data for a new participant).

ID #:   Enter the participant's 7 digit CHS ID number and verify that it is correct. If you enter it in error, use the backspace key to correct it.

Note: If you are not testing a participant, use 999 as the first 3 digits of the ID number.

Date: Verify that the computer knows the correct date.

Location: Your Field Center's name should be here.

Age: Enter the participant's age.

Sex: Press M for male or F for female.

Height : Enter the participant's measured standing height (in stocking feet) in inches.

Weight: Enter their weight in pounds. If computed BMI exceeds 27, you will be instructed to ask the participant to stand during spirometry maneuvers.

Race: Enter the ethnic code: N for Native American, A for Asian, B for Black, C for Caucasian, H for Hispanic

Note: Predicted values for Asians and Blacks are reduced by 12%, due to a shorter trunk to height ratio.

Baro: The average barometric pressure at your location (usually between 720 and 760) should be displayed here. It should NOT be changed.

Temp: The spirometer temperature is measured by an internal sensor and displayed here. Verify that it reads within 2 degC of the small MICRONTA thermometer mounted on the spirometer.

If the readings differ by more than 2 degC, call the PF Reading Center. If the spirometer temperature is below 17 degC (60 degF), the room is probably too cold for testing. Turn up the room's thermostat and blow into the spirometer yourself to warm it before testing participants.

Help. Each entry is verified to make sure it is within a reasonable range. If your entry is rejected, press the F1 key for a help message which explains the entry expected.

Editing. If a mistake was made when entering information, use the arrow keys to move the cursor to the error. Then begin typing the information. Press ENTER to complete the line.

The predicted PF values will be displayed in a box in the lower right hand corner of the screen. Ignore them and press the Enter key.

A comments screen is displayed next: Press the Enter key twice to skip over the two lines of general comments. (You will get a chance to enter these just before you print the report.) Indicate if the participant will stand for the maneuvers due to a large body mass index (above 27). Enter your 3 digit tech ID number (otherwise you will not be credited with high quality testing!) Press the Enter key at the bottom of the screen to return to the MAIN MENU.

2.8   Forced Vital Capacity Testing

You, the technician, are the critical part of the pulmonary function testing system, since you must guide the participant through breathing maneuvers which are highly dependent on participant effort. You must coach the participant to inhale maximally and then to exhale maximally. You also must judge the quality of his effort. To obtain accurate results, the testing must be done in a standardized fashion.

Note: This manual refers to the participant as "he" or "him" for easy reading, although participants will be both ladies and gentlemen.

Wash your Hands Participants will appreciate your consideration if you make a point of washing your hands before testing them. Do this as you enter the testing room if it has a sink, otherwise, just before you enter the room. Another thing you can do to minimize the risk of cross-contamination is to store the fresh mouthpieces in a sanitary plastic box and ask the participant to use a Kleenex tissue to remove one for their use. Then allow them to attach it to the clean breathing tube.

Explain the Procedure Explain that the purpose of the next test is to determine how hard and fast he can exhale air, "Like blowing out dozens of candles on a birthday cake." Explain that, as before, he should take in as deep a breath as possible, and when his lungs are completely full, quickly position the mouthpiece as before, and exhale his air as hard and fast as possible, until told to stop.

Position the Participant Testing should usually be conducted in the **sitting position**; however, obese participants (BMI>27) should stand. A chair (without wheels) should be positioned behind obese participants who stand for the test. Use the chair if the participant becomes light-headed or faint during testing. Ask the participant to sit erect with chin slightly

elevated.

Tight clothing, such as a tie, vest, or belt, which might restrict maximal breathing efforts, should be loosened. **Dentures**, if they are loose, should be removed and placed in a clean denture cup, since they will prevent a tight seal from being formed around the mouthpiece. If dentures are not loose, leave them in place.

Always Demonstrate the Maneuver Ask the participant to watch you perform the FVC maneuver. Again demonstrate correct placement of the mouthpiece. Stand up straight. Take a deep breath, throw back your shoulders, widen your eyes, and stand on your toes to emphasize the maximal depth of inhalation. Then place the mouthpiece and dramatically **BLAST** out all of your air as hard and as fast as you can.

Your vigorous demonstration will prevent time and effort from being wasted on unacceptable forced expiratory efforts which are caused by the participant's failure to understand a verbal explanation of the procedure.

FVC Test Steps

Step 1 From the MAIN MENU, select **FVL**. Move the silver lever to about the 1 liter position — the FVC Incentive screen will then be displayed.

Step 2 Tell the participant to "take in as deep a breath as you possibly can, then put the mouthpiece in your mouth." Watch him as he does so and then coach him: "now inhale a little bit more," until you are sure that his lungs are full.

Step 3 Shout "**BLAST OUT !!!**
Lower your voice a bit and say "keep going ... keep on pushing out all that air... a little bit more ..."

Step 4 After a couple of seconds, the tail of the flow-volume curve will be displayed in a box in the upper right-hand corner of the screen. Glance at it. Perhaps draw his attention to it and the horizontal bar. You will hear a beep when the EOT criterion is met, but keep coaching him to keep blowing out the air until only the green portion of the EOT plateau bar is showing (or 15 seconds has elapsed).

Watch the body language of the participant as he attempts to follow your instructions. **Pay attention to him, not the instrument.**

Encourage him to blow out smoothly without re-breathing.

Don't press the Esc key during testing until you are certain that you have performed enough good maneuvers. Press the spacebar to get the results screen. (You'll have to press it twice if you

didn't wait for 15 seconds to elapse.) Then save the maneuver by pressing the spacebar again. If you press the N key at this point, the maneuver will be erased forever. Do this *only* if the maneuver was terrible and you are sure that the participant can do a better maneuver. Analyze the flow-volume curve produced by this maneuver. Note the maneuver quality message in the box.

Hint: If you like traditional volume-time spirograms, you can display them by pressing the F8 key at this time.

If after the initial demonstration, the participant fails to perform the maneuver correctly, **again** demonstrate both the error and the correct performance yourself. You may have to repeat the demonstration after **every** maneuver for some participants!

Your goal is to obtain at least **3 good** maneuvers, 2 of which match each other closely. If the current maneuver did not match the best prior maneuver, a message like "Next time, take a deeper breath" will be displayed at the bottom of the screen.
Quality grades from A-D will be displayed immediately after the FVC and FEV1 results. These indicate the reproducibility of the best and second best maneuvers.
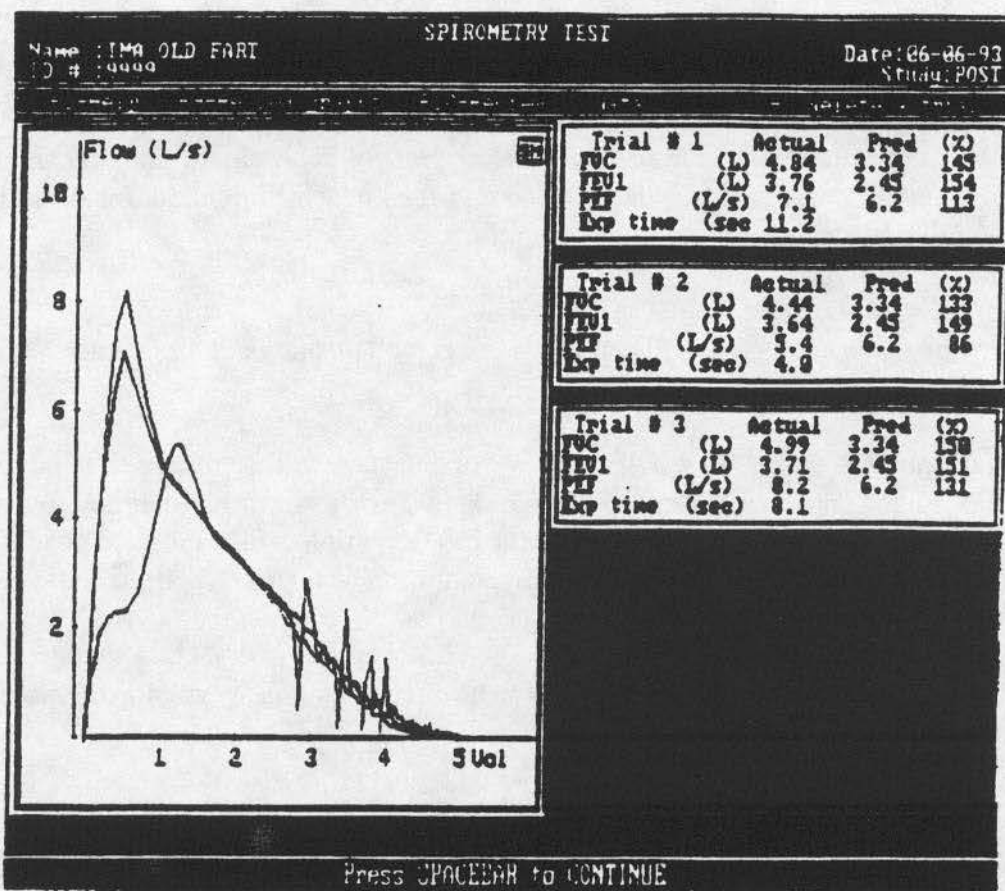
Note: Try using a noseclip if you get the message "Deeper breath" indicating that the FVCs do not match.

To perform another maneuver, merely press the Spacebar and move the lever back to the 1 liter position to get the incentive display.

Review the Results

After the participant has performed three apparently good FVC maneuvers, review the results. **Press the F9 key** to see the three best maneuvers superimposed, each in a different color.

The blue maneuver with numeric results listed at the top right of the screen in blue is the "best" maneuver obtained so far. The Trial number is the order in which it was performed.

The "best" maneuver is the one with the highest sum of FVC + FEV1. Ignore the predicted and %predicted values displayed in the right-hand columns.

If you still don't have 3 good maneuvers, press the Spacebar twice to perform another maneuver. If the quality and reproducibility of the 3 maneuvers displayed looks good, and you think that you might be done testing, press the F10 key.

Maneuver Quality Review Window (F10)

The best three maneuvers are again indicated at the top of the columns. First look at the bottom row marked QC. Any letters there are maneuver Error Codes which mean that the maneuver was not acceptable or reproducible, and that more maneuvers should be performed. Press the F1 key for an explanation of these codes. Press the Spacebar twice to resume testing.

Numbers listed under the Stored Values column are the highest obtained from all maneuvers performed and will be printed on the report. The number listed under the (%) column for each maneuver (Trial) is the percent of the highest value. For the FEV1 and FVC parameters, a good match is 95% or more. For PEFR, a good match is 85% or more.

If all 3 maneuvers are "Good tests", you have obtained enough FVC maneuvers, and should press the Esc key to store the results. The hard disk light will illuminate as the results are stored, and you will be returned to the MAIN MENU.

FVC Maneuver Acceptability

According to the ATS standards, you should coach every participant to obtain at least three maneuvers that are "acceptable" and two that are "reproducible." The criteria for acceptability and reproducibility are described below. The accuracy of results depends much more on the quality of

the maneuvers than on the instrument calibration.

Acceptability Messages Errors in FVC maneuver performance are identified by the computer and displayed in the F10 QC box:

QC	Message	Criterion
S	Start faster	BEV > 5% FVC
P	BLAST out harder	PEFT >90 msec
C	Avoid coughing	>50% drop
T	Blow out longer	FET < 6 sec
A	Blow out more air	Abrupt termination
V	Try for 10 seconds	40 ml in last 2s

After the first maneuver, reproducibility messages are also displayed on a line at the bottom of the screen prior to the next maneuver if the current maneuver's result was lower than the previous highest value from an acceptable maneuver:

d	Deeper breath	dFVC > 5% and 200 mL
f	Blow out faster	dFEV1 > 5% and 150 mL
h	Blow out harder	dPEFR > 15% and 1 L/s

Notes: QC = error code displayed in the Review QC window - F10 key.

BEV = back extrapolated volume

dPEFR, dFVC, dFEV1 = difference between the current maneuver's value and the highest value from any other acceptable maneuver from the testing session

Maximum Number of Maneuvers. Don't exhaust the participant by asking them to perform more than **eight** FVC maneuvers. If you haven't obtained 3 acceptable maneuvers by the time you have done 8 maneuvers, it is unlikely that you will. Make a note of the reason why the participant couldn't perform the maneuvers well in the Comment Screen later.

The following figures show examples of flow-volume curves from acceptable and unacceptable maneuvers.
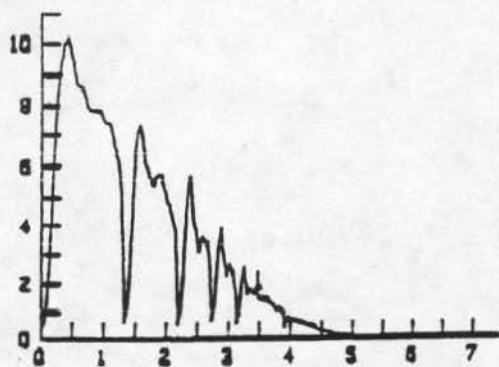
EXHALED VOLUME (LITERS, BTPS)

AVOID COUGHING
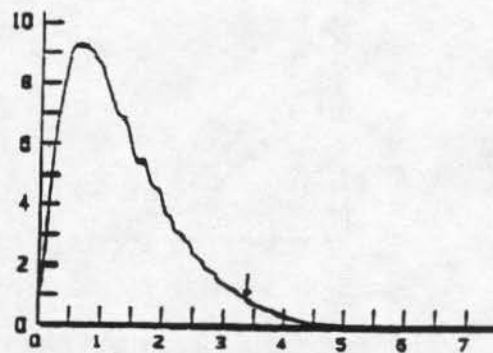
EXHALED VOLUME (LITERS, BTPS)

GOOD MANEUVER
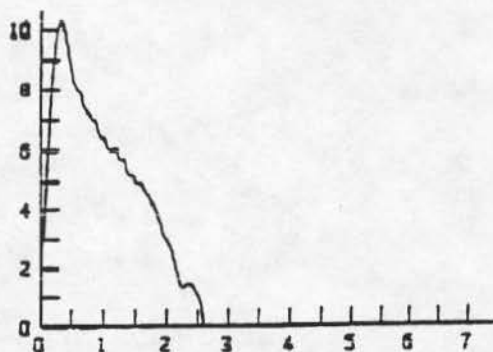
EXHALED VOLUME (LITERS, BTPS)

BLOW OUT LONGER
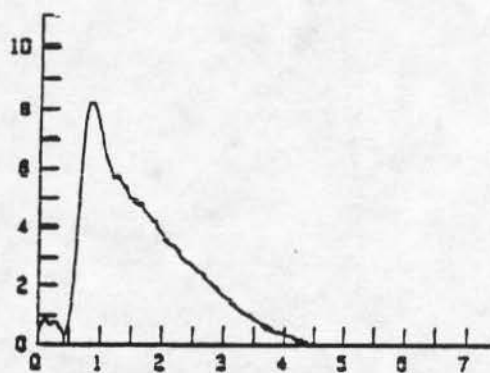
EXHALED VOLUME (LITERS, BTPS)

START FASTER
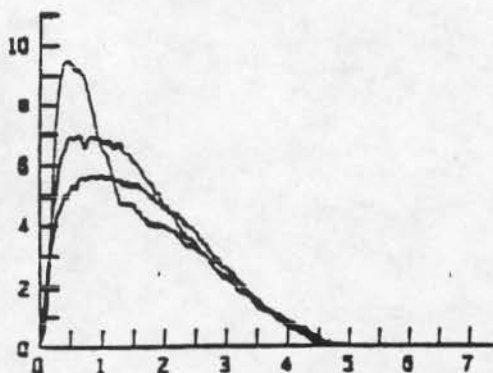
EXHALED VOLUME (LITERS, BTPS)

BLOW OUT HARDER
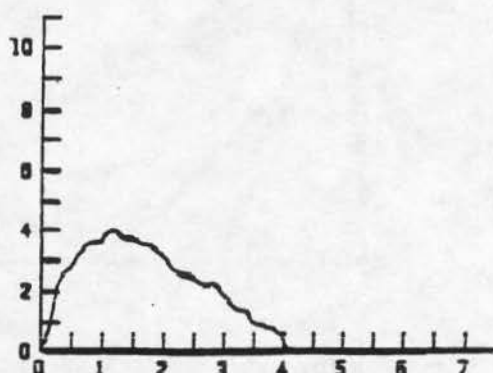
EXHALED VOLUME (LITERS, BTPS)

BLAST OUT HARDER

EXHALED VOLUME (LITERS, BTPS)

TAKE A DEEPER BREATH

EXHALED VOLUME (LITERS, BTPS)

BLOW OUT FASTER

EXHALED VOLUME (LITERS, BTPS)
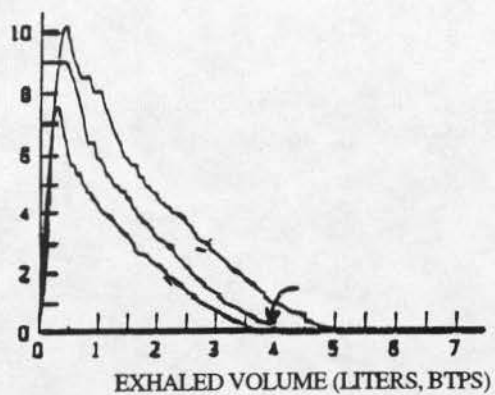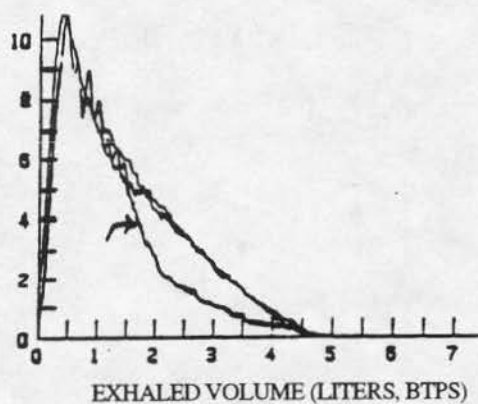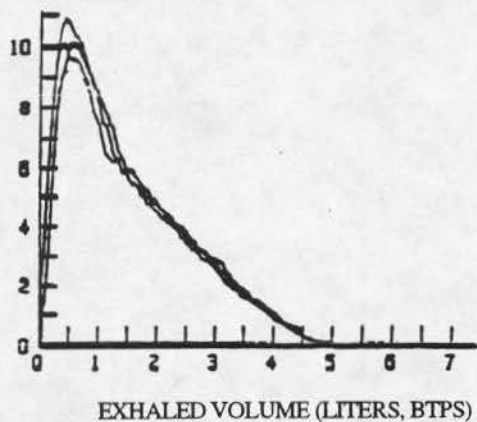
THREE GOOD MANEUVERS

2.9   Slow Vital Capacity Testing

Participants who are unable (or unwilling) to perform three acceptable forced vital capacity maneuvers should be asked to perform two easy slow VC maneuvers. Select SVC.

Demonstrate the SVC Maneuver

Ask the participant to watch you perform the SVC maneuver. With an extra cardboard mouthpiece, not connected to the spirometer, demonstrate the correct placement of the mouthpiece. Stick out your tongue and place the mouthpiece on top of it. Then withdraw your tongue, pulling the mouthpiece inside of your mouth, and seal your lips around the mouthpiece. Breathe normally for a few breaths, then take a deep breath, throw back your shoulders, widen your eyes, and stand on your toes to emphasize the maximal depth of inhalation. Then slowly exhale all of your air for several seconds.

Sample SVC tracing:
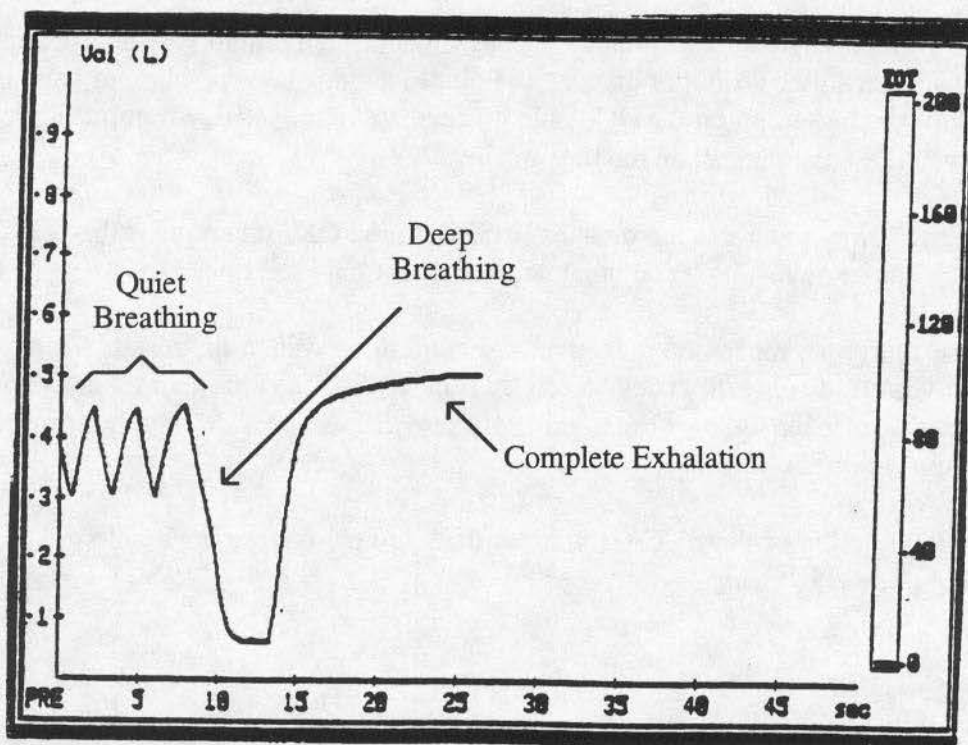
SVC Maneuver Steps

1. Move the silver lever to about the 7 liter position. Ask the participant to hold their nose during the SVC maneuvers. Attach a **noseclip** only if you notice that the participant is leaking air through his nose during the maneuvers or if you cannot obtain reproducible results.

2. Instruct the participant to seal their lips around the mouthpiece and breathe normally from the spirometer. Press the spacebar to begin the test when they have begun breathing from the spirometer.

3. Note the blue tracing of their breathing pattern starting on the left side of the screen. Allow him to breathe normally for a couple of breaths. Then coach him to take as deep a breath as possible. Look at him to see if he is doing so. Tell him to strain to take in a little bit more air.

   (SVC steps continued)

4. When you are sure that he cannot inhale any more air, tell him to let it all out slowly and then squeeze all the air out of his lungs. Point to the display. Tell him to keep blowing out until the bar graph on the right side of the display moves down into the green area (and you see a flat plateau on the blue tracing).

5. Press the Y key to accept the maneuver if it seemed OK. Then press the Enter key to view the numeric results. You don't need to adjust the FRC line.

6. After a short rest, repeat the maneuver a second time. When the results for the second maneuver are displayed, check to see that the SVCs from the two maneuvers match within 5% of each other. Press the F10 key to see the SVC result from all SVC maneuvers done so far.

7. After completion of the SVC tests, press the Esc key to store the results and return to the MAIN MENU.

2.10   End Test Session

After you have performed all of the maneuvers, congratulate the participant for a job well done and tell him that the results will be explained to him at the end of the visit. Do not attempt to explain them to him yourself.

Get the printer ready to print the report.

Select "EOS - End test session" from the MAIN MENU. The results will be added to the patient directory and database on the hard disk.

You will then be asked if you have any comments. If anything unusual happened during the testing, enter your comments on the two lines provided.

The reports will then be printed (see samples on the next ppages).

2.11   Print-Screen

Anytime while you are testing a participant and you wish to make a copy of what is displayed on the screen, you may do so by pressing the <Print Screen> key located in the upper right-hand corner of the keyboard. A box will then be displayed near the bottom of the screen asking if you want a Small, Medium, or Large size print. Normally you should select a small print by pressing the S key. This will allow two such screens to be printed on a single sheet of paper.

To eject the page from the printer, following a Print-Screen, you may need to take it "off-line" then press the Form Feed button, wait for it to eject, then press the On-line button again. Sample report printed for the participant (or their private physician):

2.12   Leak and Calibration Checks

Leak Check   Select "LEA - Leak Check" from the QC column of the MAIN MENU. The leak test must be performed BEFORE the Volume Cal Check, since a leak will affect the volume calibration.

1. Attach a breathing hose. Raise the spirometer bell by the silver lever knob to the 7 liter position. Cork the breathing hose with the #8 rubber stopper.

2. Attach the rubber band to the silver lever in order to provide pressure inside the spirometer. [A 3 inch long 3/8in wide rubber band stretched to the 7 liter mark will provide 2 cm H20 pressure inside the spirometer, per ATS specifications.]

3. Press the Enter key to start the leak test (for the default 30 seconds).

The Leakage Rate displayed after one minute should be less than 40 cc/min (or blank, indicating no leak at all).

If a Leak is detected, the message "unacceptably high leak rate ... " will be displayed. Determine whether the leak is in the breathing tube or a spirometer seal as follows:

1. Disconnect the breathing tube from the spirometer. Raise the lever midway and insert a #8 solid stopper into the breathing tube connector at the front of the spirometer. Attach the rubber band again.

Sample report printed for the participant
(or their private physician):

Cardiovascular Health Study
Pulmonary Function Report

Patient:	Martin McInroe	Height:	77.0(in) - 196 (cm)	Sex:	M
ID Number:	1233	Weight:	170(lb) - 77 (kg)	BMI:	20.2
Date:	06-11-93	Age:	45	BP:	760
Temp:	23	ATPS:	.919		
Clinic:	Arizona				
Predicted:	Knudson 83				

		Actual	%Pred	Pred
FVC	(L)	5.44	85	6.38
FEV1	(L)	4.16	80	5.18
PEF	(L/S)	10.5	97	10.8
FEV1/FVC	(%)	76.5	94	81.1

Comments:

Good Test
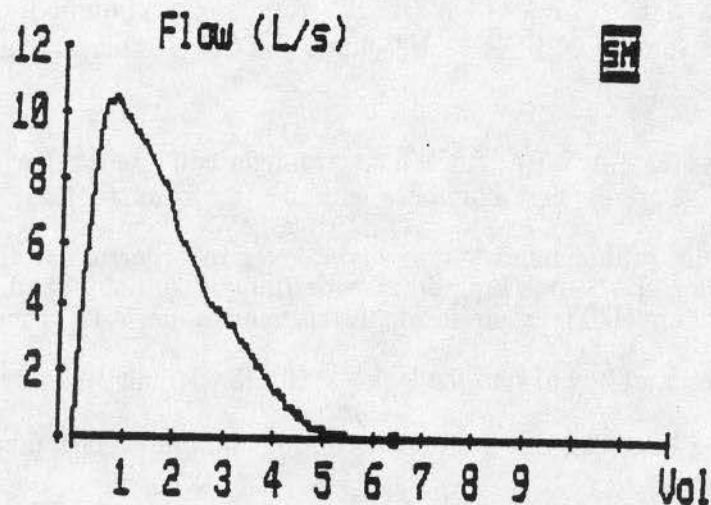
Computer Impression:

SPIROMETRY is within NORMAL limits.

Sample report printed for the participant
(or their private physician):

Cardiovascular Health Study
Pulmonary Function Report

Patient:	James Garner	Height:	70.0(in) - 178 (cm)	Sex: M
ID Number:	12376	Weight:	190(lb) - 86 (kg)	BMI: 27.3
Date:	06-11-93	Age:	54	BP: 760
Temp:	22	ATPS:	.918	
Clinic:	Arizona			
Predicted:	Knudson 83			

		Pred.	Selected	Trial 1 Actual	(%)	Trial 2 Actual	(%)	Trial 3 Actual	(%)
FVC	(L)	4.62	5.60	5.33	115	5.60	121	5.27	114
FEV1	(L)	3.73	4.09	3.99	107	4.09	110	3.83	103
FEV1/FVC	(%)	80.9	73.1	74.9	93	73.0	90	72.7	90
PEF	(L/S)	8.8	11.8	11.7	132	11.8	134	12.5	142
Exp time	(sec)		12.0	11.9		12.0		14.1	
PEFT	(sec)		0.06	0.06		0.06		0.05	
BEV	(mL)		91	92		91		60	
SEQ#		6	2	1	17	2	35	3	52
QC code		32			32				

Flow - Volume Loop best trials

Best Slow Vital Capacity
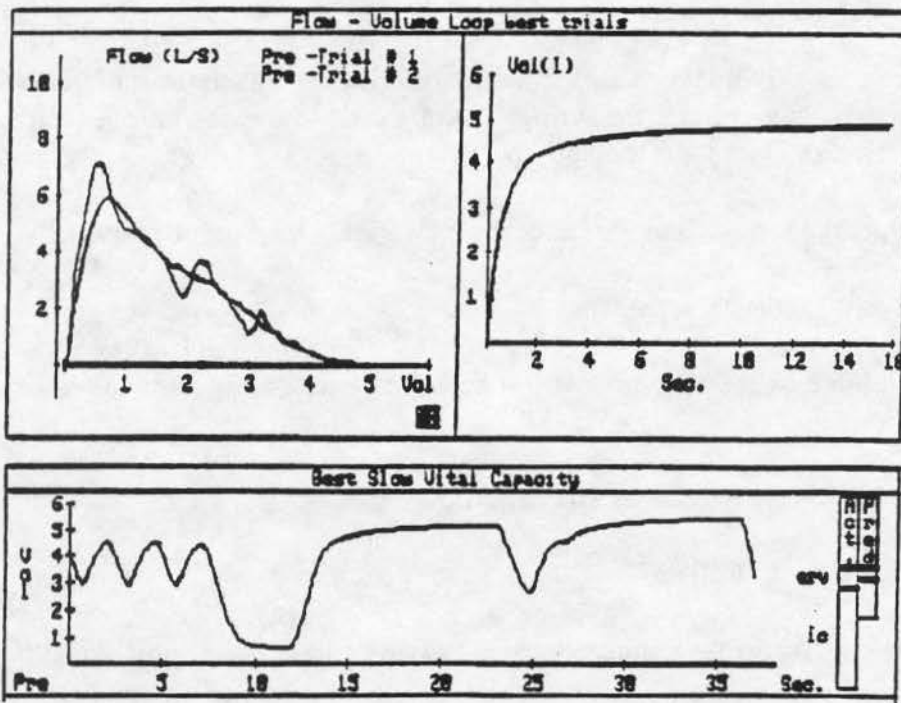
2. Repeat the leak test. If the leak is gone, then the breathing tube was the source of the leak. Discard it and check the new one for leaks. If, however, the Leakage Rate is still larger than 40 cc/min, a seal inside the spirometer is probably leaking.

3. Snap the top off the spirometer and examine closely the large black rubber "O" ring on the base. Is is seated in the round groove? Is it cracked or worn? Try applying a thin layer of some stopcock grease to the "O" ring and carefully resealing the spirometer, then see if that s=fixed the leak. If not, call S&M Instruments for help.

Volume Cal Check

Select "CAL - Volume Cal Check" from the QC section of the MAIN MENU. You should have first done a leak check. You'll need the 3.00 liter calibration syringe.

Carefully follow the directions at the bottom of the screen.

1. Make sure that you have stored the 3.00 liter calibration syringe very close to the spirometer so that they remain at the same temperature. Flush the syringe and the spirometer at least 3 times with room air. Detach the white mouthpiece adaptor.

2. Pull back on the syringe plunger until it clicks (thereby filling it completely with room air).

3. Move the silver lever to the 2 liter mark. Firmly attach the calibration syringe to the breathing hose. Place the syringe flat on the table and don't move the tubing during the next step. Then press the Spacebar.

4. Empty the syringe into the spirometer; then press the Spacebar again.

5. Disconnect the cal syringe.

If the volume calibration error is too high (more than 2%) press the Y key to re-run the volume cal check.

Press Enter to return to the MAIN MENU.

If the Volume Check Fails

Possible reasons for the volume check to fail (in order of decreasing likelihood) include:

- Failure to completely fill and/or discharge the syringe into the spirometer. Make sure the syringe clicks against the stops with each stroke.

- Differences in the air temperature between the spirometer and the syringe. Reflush and repeat the check.

- An air leak in the calibration syringe. Fill the syringe, plug the end with the rubber stopper and try to empty the syringe. If the plunger moves inward, this indicates a leak in the syringe seal. Call the PF Reading Center to replace the syringe.

ADJ If the volume error was greater than 2% during the calibration check, you will be instructed to try the above 5 steps again. If the error remains too high, you will be instructed to adjust (ADJ) the A/D converter calibration constants by carefully following the directions at the bottom of the screen.

Note: Stroke the syringe in and out completely at least three times. Take about one second for each stroke. End up with the syringe completely full (shaft extended). Make sure you hear it click at the end of each stroke, but don't "bang" it too forcefully.

2.13   Cleaning the Spirometer

Clean the Breathing Tubes at the end of each day of testing. First wash them in warm soapy water, rinse, roughly dry, then soak them in the disinfectant solution for at least 30 minutes. Be sure to wear protective rubber gloves (and a respirator?) when using this disinfectant since it causes a rash in some persons and the fumes are irritating. Rinse thoroughly and hang them to dry completely overnight before reusing.

Clean the Spirometer every Friday afternoon.

1.   Unplug the spirometer power cord from the rear of the spirometer and disconnect the serial interface cable leading from the the spirometer to the rear of the computer. Detach the breathing tube.

2.   Unlatch the top of the spirometer from the base using the four silver thumb latches. Lay the top on its side.

3.   Wash the base of the spirometer and wipe the inside of the breathing tube connector with a mild detergent solution, rinse it with water, and allow it to dry overnight before re-assembly.

4.   Reassemble the spirometer and latch the top to the base. Repeat a leak check before using the spirometer.

Calibration Syringe Care

The 3.00 liter calibration syringe should be stored next to the spirometer so that it remains at the same temperature as the spirometer. Store the syringe with the plunger pushed all the way in. Take care not to drop the syringes.

DO NOT attempt to make any adjustments to the syringe. Do not loosen the metal rings on the shafts, since this will spoil the factory calibration. The accuracy of each syringe will be verified by returning it to the manufacturer for measurement of its water displacement at the beginning of the last year of testing or whenever any evidence of physical damage to the syringe is noticed.

You should periodically check each syringe for leaks. Fill it with air, hold your palm against the outlet snout, and try to empty it. If you can expel any air with the outlet plugged, the syringe has a leak and must be repaired.

2.14 Tech Certification

The certification examination includes 40 multiple choice questions based on this Manual of Procedures, and a practical demonstration of skills including leak and calibration checks, cleaning, and testing of a naive subject (50 points). A passing score of at least 65 points is necessary for certification. Only certified technicians will perform pulmonary function testing in this study.

Certification of new technicians after the initial central training session may be performed by a centrally trained, certified PF technician. The written exam will be administered locally, and the first 20 PF tests performed will be observed by a certified PF technician and then examined by the PF Reading Center and found to be satisfactory before the new technician is certified. The results of the first 50 spirometry test sessions performed by each technician will be closely examined at the PF Reading Center. Copies of suboptimal quality test sessions with comments for improvements will be mailed to the technician the same day as they are evaluated.

A site visit to the clinical center may be made early during recruitment. Complete calibration, leak, and complete PF testing of at least three participants by each PF certified technician will be observed. Copies of suboptimal quality test sessions will be reviewed. More efficient methods as well as protocol violations will be discussed during the site visits and later in a written report.

2.15 Quality Control

Need for Spirometry QC. Examination of spirograms from the Framingham study revealed that more than 18% were of clearly unacceptable quality (11). Two more recent studies, with over 12,000 adults each, found that 40 - 50% of the spirometry maneuvers were of unacceptable quality (12-14). Manual measurements from spirograms are tedious and prone to

error (15), and deviations in test performance and lack of regular leak checking and calibration can result in loss of study data (16-18).

The Epidemiology Standardization Project (19), the new American Thoracic Society spirometry standards (20), and recent evaluations of commercially available spirometers emphasize the importance of spirometry quality control procedures. Factors which affect spirometry quality (22) include:

1. Participant
2. Maneuvers
3. Technician
4. Equipment
5. Analysis

Feasibility of QC Procedures. This spirometry system has been developed and validated by an unbiased University testing program (21). The software assists the pulmonary technician with quality control of maneuvers, calculates the PF variables, suggests interpretations, formats and prints reports, and compresses graphics data for transmission and archival storage (23). The Lung Health Study (24), Cardiovascular Health Study, Framingham Study, and ARIC studies have used similar systems and procedures since 1987. The computerization of spirometry QC procedures dramatically decreases the overhead time associated with spirometry testing.
Implementation of QC Procedures. There are five separate levels of quality control implemented for spirometry testing which address the five factors known to influence the results:

1. Daily spirometer leak and calibration checks using a 3.00 liter syringe as the "gold standard" check the **equipment** accuracy.

2. Eight computerized checks of FVC maneuver acceptability and reproducibility check **every maneuver** immediately after it is performed.

3. The PF technician is trained to recognize the patterns of unacceptable maneuvers, **watching the participant** during the performance, and reviewing the colorfully displayed flow-volume curves on the computer monitor.

4. The results of the leak and calibration checks and of the best 3 FVC maneuvers are stored and sent to the PF Reading Center for review by the PF QC Supervisor. Monthly reports are compiled for each **technician's performance**.

5. Results from all of the above are taken into account during the **analysis** of the data by the PF Reading Center (3,24). The calibration factors, PF tech's impression of participant and maneuver quality, and the QC supervisor's impression of test session quality are all integrated to obtain the final FEV1 and FVC results reported to the Data Coordinating Center.

6.	**Replicate testing** will be performed on a total of 30 participants scattered throughout the recruitment period. Choice of the participants will be by the Field Center staff, usually a participant who did not complete an exam and must return on another day to finish it. Spirometry should then be performed again by a different PF technician. The PF reading center will then examine the two sets of results for reproducibility.

7.	After instrument QC checks, a **biologic control** subject (nonsmoker without asthma) will be tested each Monday morning (the Field Center Supervisor is preferred). The results will be compared with their prior mean values for FVC and FEV1.

Weekly Biologic Control

Type GET from the MAIN MENU. Use the same technician and the same ID number for all tests. It should be 999xxxc where xxx is the tech's 3 digit ID code and c is the appropriate check digit. Press Enter to skipp all the comments. Perform FVC maneuvers as if testing a participant. Store the results and then review the trends by selecting TRD from the MAIN MENU. Ensure that your current FEV1 is within %5 of the mean of your previous values.

2.16	QC Analysis and Reporting

Each week you will mail a diskette to the PF Reading Center, using the BAK command which copies all the spirometry results for participants tested during the previous week onto a floppy diskette.

At the PF Reading Center, the result files are read by the PF QC workstation. The PF QC workstation displays the 3 best FVC maneuvers from a test session as differently colored flow-volume curves superimposed at the onset of each maneuver. The best maneuver is marked "B". The color of the maneuver sequence number (#1-8) corresponds with the color of that maneuver's flow-volume curve. The peak expiratory flow (PEF), FEV1, forced expiratory time (FET), and forced vital capacity (FVC) follow.

The Field Center and the PF technician who performed the testing are hidden from the QC Supervisor to avoid bias. The spirometer temperature is displayed and is highlighted if it falls outside the 17-33 degree C range, since BTPS corrections for volume spirometers become less accurate outside of this "normal" range (27).

After evaluating the flow-volume curves and the array of results, the QC supervisor indicates her choice of the single best maneuver, and enters a test session QC grade from A to F for both flow and volume. The flow grade is an index of reliability of the FEV1 from that test session. A flow grade of **A** is entered if at least 3 maneuvers demonstrate sharp PEFRs and if the best two have very reproducible PEFs and reproducible FEV1s (28).

The volume grade is an index of reliability of the FVC. A volume grade of **A** is entered if at least 3 maneuvers have maneuver durations of at least 10 seconds and the best two have very reproducible FVCs. A test session which just meets the minimum ATS recommendations of 3 acceptable maneuvers with the best two reproducible within 5% will generally receive a flow and volume QC grade of **B**.

After overreading a batch of test sessions, the QC grades are added to a QC database. All sessions with either a volume or flow grade of **C** or less or with a spirometer temperature outside the normal range are printed, comments are added by the QC Supervisor, and a cover letter is added and mailed to the technician who performed the test. The final, overread PF results are generated and sent by mail to the Data Coordinating Center at least monthly.

At the end of each month, a report is generated from the QC database, summarizing the performance of each PF technician. For each PF technician, the report includes the number of sessions reviewed and their average QC grades. The report is mailed each month to the Principal Investigators and to all PF technicians.

2.17   Annual Instrument Checks

Prior to the onset of the study, and at least annually thereafter, the following items will be checked to ensure spirometer accuracy:

1.   **Spirometer temperature sensor accuracy** - A thermometer accurate to within 0.1 deg C is placed inside the spirometer bell and allowed to equilibrate for an hour. The temperature displayed by the spirometer on the INF screen is then compared with it. If there is more than a 0.3 deg C discrepancy, the correct temperature is entered by using the up arrow to move the cursor to the temp box and entering the correct temperature. The new temp cal factor is then noted using the EQU command.

2.   **Volume linearity** - The linearity of the spirometer throughout its volume range is checked using a 1.00 liter calibrated syringe with internal one-way valves (Vitalograph). The LIN command invokes a program which directs the operation of this check. A worst-case linearity of 0.2% is the threshold of acceptability.

3.   **Chart motor speed** - According to ATS recommendations, the chart motor's speed of 20 mm/sec should be accurate to within 1% to allow accurate manual calculations of the FEV1. This is verified by drawing two lines exactly 20 cm apart on the chart paper. A stopwatch is started and stopped as the pen passes the marks. This should be repeated a couple of times since eye-hand coordination often results in errors of more than 1%. The average elapsed time should be between 9.99 and 10.01 seconds.

4.   **Calibration syringe volume and leak test** - The volume of the calibration syringe is checked by filling it with water, then emptying the water into a calibrated volumetric flask

or cylinder. It is checked for leaks by pressurizing it while stoppered, as described previously.

5. **ATS waveform calculation accuracy** - The 27 standard ATS spirometer waveforms are available from S&M Instruments on a disk. These are "played into" the software (bypassing the A/D converter) to verify the accuracy of the software's calculations by comparing them to the published results. This check, however, doesn't check the spirometer or A/D converter nor the BTPS corrections.

2.18 References

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2.19   Appendices

EQUIPMENT AND SUPPLIES

Attach the spirometer cable to the computer with the two screws on the connector, otherwise it will fall off easily. Attach the printer cable to the rear of the PC. Attach all power plugs to the switched outlet strip.

PF Workstation Major Components

1. Dry rolling seal spirometer with internal A/D converter
2. Toshiba T1850 laptop computer
3. Color VGA monitor (optional)
4. Canon BJ-200 bubble jet printer
5. Tamarac 3.00 liter calibration syringe
6. S&M Instruments Pneumocheck II software

Spirometry Supplies

The maintenance and supplies kit includes:
   Mouthpieces, 1 3/8 dia cardboard (qty 1000)
   Sanitary storage box for mouthpieces
   Noseclips
   Breathing hoses, 36" long (qty 20)
   Diskette Holder and 10 Diskettes, 3.5 inch
   Power strip with 6 surge protected outlets
   Denture cups
   Rubber stopper #8 size for leak checks

Other supplies to be purchased locally:
   Detergent and hose cleaning bucket
   Disinfectant solution (Cidex, Metracide, etc)
   Alcohol wipes, Cleaning cloths, Q-tips

PROGRAM FILES

The following files are distributed on 3.5 inch HD floppy diskettes and initially installed on the hard disk (Drive C:) in the subdirectory C:\CHS.

DIRINF.EXE	The MAIN MENU shell program
FVL.EXE	FVC testing program
INF.EXE	Demographic information entry and calculation of predicteds
INF.REG	Predicted equations (text file)
DIS.EXE	Data File Management program
DISA.EXE	More data file management
ADJ.EXE	Recalibration of volume and flow
CONFIG.DAT	Custom configuration data

The *.EXE files are compiled using Quick BASIC version 6.0.
Files with a .TXT extension are ASCII text files used to customize each program module.
*.HLP files include the text in boxes displayed when the F1 key is pressed for help.

The software version number displayed at the top of the MAIN MENU is coded as follows: Ver. MMY.CCA.XX, where MM=month, Y=last digit of year, CC= Microsoft BASIC compiler version, A=major software version, and XX=the version of minor modifications.

RESULT FILES

At the end of each test session, the results for that single participant are stored on the computer's hard disk in the subdirectory C:\PD93 as the following files:

Filename	Description of contents (type)
FILE	Participant directory (A)
DATAxxxx.BST	3 best FVC maneuvers + 8 parameters (G)
DATAxxxx.LOP	1 best FVC maneuver (G)
DATAxxxx.MIP	5 best MIP maneuvers (G)
DATAxxxx.MEP	5 best MEP maneuvers (G)
DATAxxxx.TXT	Comments and other free text (A)

(A) = ASCII file, (G) = Binary Graphics array

xxxx is an internal sequence number, unique for each participant's test session, starting from 0.

The total size of these 8 files for one participant is about 8 Kbytes. A maximum of 800 participants can be stored in a single PD subdirectory, but additional test result subdirectories may be created (the subdirectory name must start with the prefix PD, short for "Patient Directory").

The file confusingly called FILE includes each participant's name, ID number, and test date.

Every time a BAK command is performed, all "unmarked" files in the PD93 subdirectory are copied to Drive A: (but not deleted from the PD93 subdirectory). They are then "marked" as having been copied.

Two large database files also exist in the PD93 subdirectory: **DATA.DOC** is a redundant database file which contains the numeric results from the best single FVC maneuver for all participants ever tested on the workstation. **INTERP.DAT** is a large empty database file which enables other users to create and store multiple lines of free text comments or interpretations (in addition to those stored in the individual DATAxxxx.TXT files) for each participant. The CHS will not use the INTERP.DAT file, but it cannot be deleted.

QC FILES

An ACII file called SPIRO.LOG is also located in the PD93 subdirectory. It stores the results of CAL checks. It is formatted so that it may be printed using a simple DOS copy command on 8.5 x 11 inch paper. A comments line follows each cal check record. The Adjustments columns are used only when a recalibration (adjustment) was performed. C=A/D Channel number, V=Volume gain factor, F=Flow gain, I=MIP gain, E=MEP gain.

Temporary result files. Several result files are created temporarily during test sessions, but are overwritten whenever a new participant is selected by the INF program:

> PRE.BAS
> COM.DOC
> PTA.DOC

The PTA.DOC is an ASCII file which contains the information necessary for the participant's summary report. When FIN is selected from the MAIN MENU, PTA.DOC is copied to a file called yyyyyy.PU on the E:\DATA subdirectory of the print station's hard disk, where yyyyyy is the CHS participant ID number.

CUSTOM CONFIGURATION

SMI's commercial software has been customized for use by the CHS. The configuration is altered by a program called CON which displays the following screen and then creates a file called CONFIG.DAT to store the results. The CON program should NOT be altered by the PF technician. However, for reference purposes, the correct configuration setting for the CHS are as follows:

COMPUTER INTERPRETATION

The Printer Workstation will compare the observed values to those predicted by the CHS baseline data from the healthy participants (27), and then interpret them based on the American Thoracic Society recommendations for disability testing (28):

Interpretation:	Criteria
Normal spirometry:	FEV1/FVC ratio $\geq 70\%$ and FEV1$\geq 80\%$ predand FVC $\geq 80\%$ pred
Borderline obstruction:	FEV1/FVC ratio < 70% but FEV1$\geq 80\%$ pred.
Mild obstruction:	FEV1/FVC ratio < 70% and FEV1 60% to 79% pred.
Moderate obstruction:	FEV1/FVC ratio < 70% and FEV1 of 41% to 59% pred.
Severe obstruction:	FEV1/FVC ratio < 70% and FEV1 < 40% pred.
Reduced vital capacity:	FVC < 80% pred, in addition to obstruction.
Mild restriction:	FVC 60% to 79% pred, with FEV1/FVC ratio $\geq 70\%$
Moderate restriction:	FVC 51% to 59% pred, with FEV1/FVC ratio $\geq 70\%$
Severe restriction:	FVC 50% or less than pred with FEV1/FVC ratio $\geq 70\%$

The software calculates predicted values using equations stored in a file called INF.REG The interpretation cutpoints and messages are stored in a file called DAT.TXT

MAINTENANCE

Serial cable. The RS-232 serial interface cable uses standard 9 pin IBM PC AT connectors. Only pins 2,3,5,8,9 are connected.

A/D Converter Check. A program to check the A/D converter channels is easily obtained by pressing **ADT** from the MAIN MENU. Nine different options are then displayed on the A/D Check menu. The most useful is "6 Check S&M Channels" which gives a continuous display of the Volume, Flow, and Temperature readings in converted units. You may then move the spirometer bell up and down and watch the Volume and Flow change.

The "7 Check all Channels (Raw)" option on the ADT menu displays the instantaneous Actual inputs in raw A/D counts from 0 to 4000:
Press the R key to reset the "Difference" column to all zeroes and the drift or noise may then be measured by observing the maximum change for each channel.

Channel 1 is spirometer Volume. It should be near zero (0-50) when the pen is on the baseline, and increase to close to 4000 when the bell is raised to 8.0 liters. If not, the potentiometer or its connections may be bad.

Channel 3 is the Reference voltage (+5 volts DC). It should remain constant at about 4000 counts.

Channel 4 is the spirometer temperature. It should be between 150 and 250 at room temperature (higher at higher temperatures).

Channels 2 and 5-8 are not used and should all read 0.

3. SKIN TESTS FOR TUBERCULOSIS AND COCCIDIOIDOMYCOSIS

3.1 Rationale:

Tuberculosis (TB) has been a scourge of Indian people for many generations and only in the last decade have the rates of tuberculosis diminished to rates that are only several times higher than the U.S. rate. Until the 1960s tuberculosis was unquestionably the most serious health problem afflicting Indian people. With the advent of effective chemotherapy and the promotion of chemoprophylaxis, tuberculosis has diminished rapidly but still occurs three to four times more commonly than in the U.S. Diabetes and chronic renal failure are known to be risk factors for tuberculosis. The rationale for performing tuberculin skin tests on the Strong Heart Study cohort is: 1) to establishthe prevalence of tuberculin positivity. 2) to refer Strong Heart Study participants with positive tuberculin tests to IHS for appropriate evaluation and treatment. 3) to establish a prevalence of history of TB.

This will be of special interest in the Pima population since they participated in a BCG vaccine trial in the 1930s. One half of the participating children received BCG and the other half received the placebo. John Hopkins University is currently doing a follow-up evaluation to ascertain the protective efficacy of the BCG vaccine as well as any potential complications especially with regard to increase or decreased incidence of cancer among those receiving the BCG. Coccidioidomycosis (Valley fever) is a pulmonary disease that is transmitted to humans through airborne organisms in the Sonoran desert environment. Thus the range of the disease is limited to Arizona and southern California. Exposure to this disease can be detected through a skin test similar to a tuberculin test. Although the disease is mild in most individuals, it can cause serious damage in some individuals. Systemic cocci disease occurs more commonly in diabetic patients and other patients with immunosuppression. The coccidioidomycosis skin test will only be done on Arizona participants.

3.2 The Procedure for Recording the Skin Test

The Procedure for recording the skin Test is as follows:

1) IHS medical records are reviewed and results of the skin tests (PPD at all 3 sites, cocci at Arizona) are recorded on the Strong Heart Study forms. Sometimes results are recorded as mm induration and sometimes the results are recorded as positive or negative.

2) All patients will be asked, "Have you ever had tuberculosis?", yes/no.

3) If yes, date diagnosed.

4) "Did you ever have a positive TB skin test (redness/ swelling develop two days

after application) yes/no.

If participant has documented positive tuberculin test, or a history of tuberculosis, it is not necessary to repeat the tuberculin test. Strong Heart Study participants who deny history of tuberculosis, do not have documentation of a positive tuberculin test and do not have a history of a positive tuberculin test, should receive a tuberculin test in conjunction with the Phase II Strong Heart Study exam. If participants give a history of a positive skin test, their medical records should be reviewed to verify results if possible. If results cannot be verified, the participants should be offered a repeart skin test. If the patients give a history of active tuberculosis, the skin test should not be repeated. The Arizona participants will be asked whether they have ever had Valley Fever. If Arizona participants have a history of Valley Fever or a documented positive cocci skin test, it is not necessary to repeat the cocci test.

3.3 The procedure for administering tuberculin skin test

The procedure for administering tuberculin skin test is as follows:

1)   Clean left forearm with alcohol swab.

2)   Draw up five tuberculin units (0.1 cc) in a tuberculin syringe.

3)   Inject 0.1cc of tuberculin, intrademally in the left forearm with the bevel of the syringe facing upward (see diagram below). Taking care not to inject near a vein.
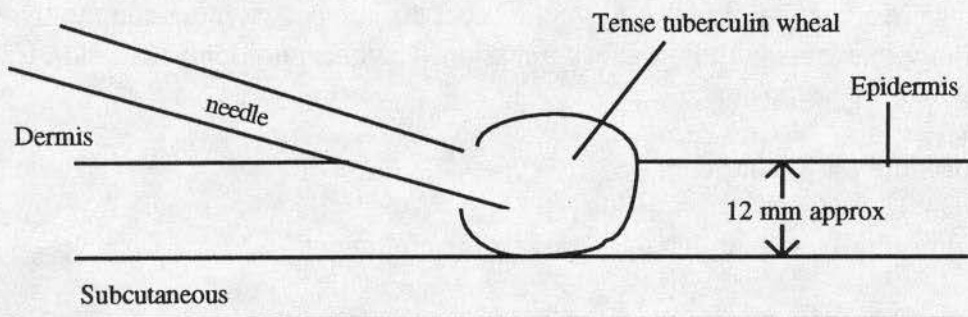
4)   This should produce a small bubble 3-4mm in diameter.

5)   Strong Heart Study staff, CHRs, or nurses who are trained and certified in reading PPD's should read the tuberculin test in 48-72 hours. The date and time should be recorded and any reactions to the test noted. The skin test should be measured in mm for the induration (raised/hard area) not the redness (10 mm or greater induration is considered to be positive) and recorded in the participants' medical

records. A tuberculin measuring device/caliper will be used to measure the induration.

6) A booster or two-step PPD is given to those participants who have not received a TB skin test in the past two years because their immune response may have waned over the years.

   a. First PPD given and read in 7 days unless the patient reacts in 2-3 days.

   b. If PPD is negative, second PPD will be given 1 week or more after the first one and read in 48-72 hours.

Participants with positive PPDs or cocci skin tests should be advised that they are at increased risk of developing TB or valley fever, especially if they have diabetes. They should seek medical care if they develop symptoms of these diseases (ie cough, weight loss, night sweats). PPD positive participants who have completed 6-12 months of preventive therapy or who have completed adequate treatment for active TB (6 or more months of therapy with 2 or more TB drugs) have a reduced risk of developing TB (about 80% reduced) Participants with positive PPDs may benefit from INH preventive therapy and should be advised to be evaluated by their health care provider if they have never received INH preventive therapy or been treated for TB. Referrel should be made if:

1) PPD positive <u>and</u>

2) Preventive therapy with INH for 6-12 months or adequate tratment for active TB has <u>not</u> been completed, <u>and</u>

3) Participant is willing to take preventive treatment if it is prescribed
                    <u>OR</u>
   If the participants have a positive PPD or cocci test and develop symptoms of TB or cocci, results of both cocci and PPD skin tests will be reported to IHS so they can be recorded in the participants' medical records.

The procedure for administering and reading the coccidioidomycosis skin test is identical to the tuberculin test except it is administered on the right forearm and should only be done in the Arizona site, since exposure to the disease is not known to occur in the other two sites.

If participants have a severe reaction to the PPD or cocci skin test, they should be advised to use hydrocortisone cream to reduce the swelling and inflammation. Rarely severe swelling and induration may occur, but this occurs more often in younger individuals and responds well to hydrocortisone.

THE STRONG HEART STUDY II

TUBERCULOSIS AND COCCIDIOIDOMYCOSIS
TUBERCULIN SKIN TEST AND COCCI SKIN TEST

ID Number ☐

A. TUBERCULOSIS AND TUBERCULIN SKIN TEST

1. History of Active Tuberculosis and Tuberculin Skin Test

    a. History of TB by medical record review :   ☐

        1=Yes   2=No
        3=Medical record not available or complete   4=Uncertain

    b. History of TB by personal interview, "Did a medical person ever tell you that   ☐
       you had active tuberculosis?"

        1=Yes   2=No   3=Uncertain

    c. If "Yes " in a or b, "what was the year of diagnosis?"   ☐☐
       Fill in year of diagnosis, 99=unknown. Skip to Section 4.

    d. If "No" or "Uncertain" in a or b, ask participant: "Have you ever had   ☐
       a positive TB skin test?"
       1=Yes   2=No   3=Uncertain

Verify PPD results in medical record and fill out Section 2 below.

2. Results of tuberculin test - *Recorded from chart review*

    a. Date of last test   ☐☐ / ☐☐ / ☐☐
                                       mo   day   yr

    b. If available, record induration (in mm). if not recorded, draw one line   ☐☐
       through the boxes.
       Comments regarding previous PPD testing: _____

    c. Interpretation:
       1=Positive ($\geq$10mm or PPD positive) (*Go to section 4*)   ☐
       2=Negative (<10mm or PPD negative)
       3=Uncertain (PPD not read)

If unable to verify positive results, offer to repeat PPD

If "Positive" in Medical Records, go to B if in AZ, or to next section if in OK or N/SD.

3.   Results of Tuberculin Test - OFFER AS PART OF SHS TO PARTICIPANTS WHO HAVE NO HISTORY OF TB <u>AND</u> NEGATIVE PPD TEST OVER 2 YEARS AGO <u>OR</u> POSITIVE OR UNCERTAIN PPD HISTORY WITH NO MEDICAL RECORD VERIFICATION

   a.   Did participant refuse the TB skin test? 1=YES, 2=NO
         If participant refused TB skin test, GO TO Section B.

1st TB test:

   b.   Date of administration (left arm preferred)

   Initial site given   right arm _____   left arm _____

   c.   Induration in mm. If unable to read skin test fill in 99.
         If <10mm induration, repeat PPD 7 days after the first test unless participant had negative skin test within the last 2 years..

   d.   Reading date

   e.   Reader's initials: _____

2nd TB test (To be given at least 1 week after the first test):

   b.   Date of administration (left arm preferred)

   Initial site given   right arm _____   left arm _____

   c.   Induration in mm. If unable to read skin test fill in 99.

   d.   Reading date

   e.   Reader's initials: _____

4. If PPD is positive or history of TB is positive, did participant complete preventive ☐
   therapy or curative therapy? (*Adequate preventive treatment is at least 6 months
   of INH. Adequate curative treatment is at least 6 months with 2 or more TB medication*)
   1=Yes   2=No (Complete a & b)   9=Uncertain

   a. If no, would participant be willing to take preventive therapy ☐
      prescribed by a medical professional?
      1=Yes   2=No   9=Uncertain

   b. Referral written for service unit follow-up? ☐
      1=Yes 2=No

If PPD is positive and the patient never completed preventive therapy or was never adequately
treated for active TB, refer for evaluation by TB control program if he/she is willing to take
preventive therapy. A chest x-ray is indicated before starting a patient on preventive therapy but is
not indicated for asymptomatic patients who have completed preventive therapy or therapy for
active TB or for those who refuse preventive therapy, unless symptoms of TB develop.

5. Coder ☐☐☐

6. Date completed ☐☐/☐☐/☐☐
   mo   day   yr

ID Number ☐☐☐☐

B. Coccidioidomycosis and Cocci Skin Test (<u>Arizona</u> participants only)

1. Results of cocci test - *Recorded from chart review*
   a.   Date of last test ☐☐/☐☐/☐☐
   mo   day   yr

   b.   If available, record induration (in mm). if not recorded, draw one line through the boxes. ☐☐
   Comments regarding previous cocci testing: _____

   c.   Interpretation: ☐
   1=Positive (≥10mm or cocci positive)
   2=Negative (<10mm or cocci negative)
   3=Uncertain (cocci not read)

2. History of coccidioidomycosis by medical record review ☐
   1=Yes   2=No
   3=Medical record not available or complete   4=Uncertain

3. Has a medical person ever told you that you had Valley Fever? ☐
   1=YES   2=NO   9=Unknown/Uncertain

Offer cocci skin test to participants who have no history of coccidioidomycosis <u>or</u> Vally Fever <u>and</u> negative cocci skin test over 2 years ago.

4. Is Cocci skin test given? (*Right arm preferred*) ☐
   1=Yes   2=No   3=Refused

   If "YES," Administration Date ☐☐/☐☐/☐☐
   mo ·   day   yr

   Initial site given: right arm_____   left arm_____

5. Induration of cocci skin test (in mm). ☐☐

6. Reading Date ☐☐/☐☐/☐☐
   mo   day   yr

7. Reader's initials: _____

Participants with history of Vally Fever or positive cocci skin tests should be advised to seek medical care if they develop fever, cough or other pulmonary symptoms. No other specific treatment is indicated.

8. Coder ☐☐☐

9. Date completed (mo/day/yr) ☐☐/☐☐/☐☐

Instructions for the Form of Tuberculosis and Coccidioidomycosis

1.   History of active tuberculosis and tuberculin skin test

The first of this section (Part A) involves medical record review for history of active tuberculosis (class III tuberculosis). Case definition for class III tuberculosis involves having a positive culture for myco bacterium tuberculosis from a body fluid or tissue or having a clinical picture suggestive of tuberculosis that responds to treatment with antitubercular medications. Information on treatment could be found on the patients problem list and discharge summaries or consultant reports that are filed in the patient's chart. If there is no evidence in the IHS medical record or other medical records that may be available, place 2 in the box. If the patient has active TB listed on a discharge diagnosis or on a problem list place a 1 in the box. If the diagnosis is suspect tuberculosis or there is uncertainty about whether the lab results meet the case definition, photocopy the information and send this for review by Dr. Tom Welty. The box number 4 could be checked in those situations until clarification is obtained.

In part B the person is asked whether he ever had active tuberculosis. Sometimes patients might be confused by a positive skin test. If the patient has questions about the interpretation of this, please probe them about whether sputum or other AFB cultures were obtained in working up the problem and whether they were positive for TB or not. If patient had TB 10 or 20 years ago, it is almost certain that they would have been hospitalized. Currently TB patients are often treated as outpatients. Patients with active TB always receive 2 or more TB medicines. Again probing about the medication is encouraged if the patient is uncertain about the TB history.

On Part C record the year (last 2 digits) of diagnosis of TB and if the information is available in the medical record this date listed there would be preferable to the patients history.

If there is no evidence of active TB by chart review or history (Part D), ask the participants whether they have ever had a positive tuberculin skin test. If the answer is NO, and if the latest recorded PPD is negative (<10 mm induration) the participant should be offered a two-step tuberculin test as described below. If the answer is YES, try to verify the results of tuberculin test through the medical record. If the results cannot be verified, offer the participant a two-step tuberculin test.

Section II requires medical record review for results of the latest tuberculin test. If the latest tuberculin test is negative, the PPD should be repeated. In some cases the latest tuberculin test will be negative but previous tuberculin tests will be positive. The tuberculin test should probably be repeated in such situations since it would be unlikely that a bad reaction would occur to the tuberculin and clarification would be needed as to the correct

interpretation of the tuberculin status which should be possible through a two-step test. This situation occurs occasionally due to waning immunity of individuals as they age. It can also occur in individuals who have previously received BCG, which we know was given in the Sacaton area, to our cohort when they were children.

2. Tuberculin Test

All Strong Heart Study participants who have no history of TB should be offered a two-step tuberculin test if they meet the following criteria:

a. Had negative PPD test over 2 years ago,

b. Positive of uncertain PPD history with no medical record verification.

Participants who have consistently had negative PPD tests recorded in their medical record with the latest results less than 2 years ago, do not need to have a repeat test done. If there is only one PPD test recorded in the medical record within the past 2 years, it would be advisable to do a repeat one-step test as part of the Strong heart Study exam. If a history is questionable or medical records are not available there is minimal risk involved in repeating the skin test and this should be done in such cases. In approximately 5% of positive skin tests, a large red reaction will occur. These reactions are self limited and gradually disappear over a period of several weeks or a month. The resolution of the reaction can be hastened by application of hydrocortisone creme 2% twice a day. Serious complications from these reactions rarely if ever occur. Since immunity wanes with age, it is unlikely that we will have a serious reactions occurring in our cohort of study participants that are all over 45 years of age.

Preventive therapy reduces the risk of developing tuberculosis in persons who have positive tuberculin tests. Recommended therapy is 6 to 12 months of INH. Once this preventive therapy is completed, no further follow-up or evaluation is necessary unless symptoms of tuberculosis develop such as cough, fever, weight loss, poor appetite, night sweats, etc. Patients with a history of active tuberculosis (Class III disease) do not need any special follow-up, if they have completed the recommended therapy. Currently therapy for active TB involves 2 or 3 medications for six months. In the past TB has been treated for 12 to 24 months routinely. Frequently duration of therapy is noted on the problem list under the problem of active tuberculosis or positive PPD and notation will be made that treatment is completed or adequate. This will help to answer question number 4 and assist you to know whether a referral if necessary. if treatment is not adequate, it will be helpful to determine whether the individual is willing to take preventive therapy. If the participant is not willing to take preventive therapy, there is no need to make a referral for follow-up unless the participant develops symptoms of active tuberculosis as described above. In the past patients with positive PPD's have been advised to have annual chest X-rays to rule out active disease. However, at present this is no longer recommended because it is not a cost

effective screening approach. If the participant has a untreated PPD and is willing to consider taking INH preventive therapy, if this is recommended by the health care provider, a referral should definitively be written. if the participant is not willing to take preventive therapy, a notation should be made on the PCC form as follows (PPD positive patient not interested in preventive therapy at the present time).

When measuring induration of tuberculin tests, it is advised that the reaction be felt with the index finger a line drawn at the edges of the induration. The induration area then can be measured in millimeters and recorded on the Strong heart Study data collection form. The two-step testing procedure involves intraderinal administration of a mantoux tuberculin test (0.1 cc) which can be read in 48 to 72 hours. A health care provider or a CHR should be trained to make these readings in a consistent manner if the initial tuberculin test is negative, a repeat test should be administered one week to one year after the first test and the result recorded. Thus persons with a initial negative PPD could be retested during the summer months by a health profession student trained to carry out this aspect of study. Results of the tuberculin test should be recorded in the patients chart on the blue immunization sheet.

3.   Coccidioidomycosis history and skin testing

In a similar manner the medical records should be reviewed for history of coccidioidomycosis (valley fever) and results of cocci skin tests. This infectious disease has clinical symptoms very similar to tuberculosis and is transmitted to humans through dust in the Sonoran desert area of the United States (southern Arizona and California). No preventive therapy is available for coccidioidomycosis so patients do not need to be referred for evaluation if they have a positive test. However, results of skin testing should be recorded on the immunization sheets in each individuals chart. Persons with a positive skin test should be advised that if they develop symptoms of cough, fever, weight loss, they should be evaluated for valley fever. Persons with a history of coccidioidomycosis or positive skin test that is verified in the medical chart do not need to have a coccidioidomycosis skin test applied.

4. ANCILLARY STUDY OF ULTRASONOGRAPHY OF THE GALLBLADDER

4.1   Introduction

This document discusses the importance of performing gallbladder ultrasonography, data collection, quality control, and data analysis.

Gallstones are a common condition and their treatment is a major medical expense. Cholecystectomy is the fifth most common non-obstetric, therapeutic hospital procedure in the United States; in 1990 there were 522 thousand cholecystectomies compared with 392 thousand coronary artery bypasses and 285 thousand percutaneous coronary artery angioplasties. Gallstones are the second most costly digestive diseases in the U.S. (behind gastrointestinal infections) with a yearly direct and indirect cost of well over $5 billion.

Gallstones are a particularly important condition for American Indians. The first ever population study of gallstones using oral cholecystography in 1967-1968 found a prevalence of gallstones among Pima Indians that was remarkably high, particularly among women. About 50 percent of all Pima Indians and more than 70 per cent of women were found to have gallstones or to have undergone cholecystectomy. This study was largely unable to define factors other than sex that were associated with gallstone disease. Since then much has been learned about the pathophysiology of gallstones, some of it from Pima Indians, but no further prevalence studies have been performed among American Indians in the United States. Besides being a common affliction, gallstones also greatly increase the risk of gallbladder cancer among American Indians. Cancer registry data have demonstrated that American Indians in Mew Mexico have the highest reported gallbladder cancer incidence in the world, more than 10 times the rate in the United States population as a whole. One study estimated that among American Indians gallstones increased the risk of gallbladder cancer 20-fold.

Ultrasonography has greatly facilitated epidemiologic studies of gallstone disease. It is safe, accurate, relatively inexpensive, and can be accomplished in a few minutes. Diagnostic criteria for gallstones are simple and have allowed standardization across studies. A great deal of experience with gallbladder ultrasonography has been gained from the third National Health and Nutrition Examination Survey (NHANES). Over 10,000 ultrasounds have been performed to date. By its completion, United States population estimates will be available for whites, blacks, and Mexican Americans. Much of the present protocol has been pulled from the NHANES because that study has been highly successful and because it would be valuable to compare the results of the two surveys.

4.2   Data Collection and Interpretation

4.2.1   Ultrasonography

Each person undergoing cardiac echocardiography will also have gallbladder ultrasonography. This examination will be performed either immediately before or after the echocardiogram. The examination protocol is as follows. Brackets ([]) indicate a feature of the NHANES protocol that may not be applicable here.

A.   Eligibility Criteria

All persons eligible for echocardiography are eligible for ultrasonography of the gallbladder. Participants should be asked to fast for least six hours prior to arriving at the clinic in preparation for the exam, but will not be excluded from the exam if they have not fasted. There are no other exclusion criteria for the ultrasound examination.

B.   Pre-Examination Procedures

1.   Check that the VCR is on and that the VCR tape has been inserted and advanced by six digits since the previous exam. [Make sure [DISPLAY] on the control panel of the main unit is off.]

2.   Code pertinent information into the identification portion of the ultrasound screen. [Press [CHAR] twice to move cursor into upper left portion of screen. Use keyboard on control panel to type in participant identification number and examiner number. Press [CHAR] once to move back to scale on screen.]

3.   Assist the participant onto the exam table and into a supine position.

4.   Ask the participant to fold up the gown top to expose the upper right quadrant of the abdomen. Drape the participant with two chucks to protect the gown top and pants.

5.   Identify the participant's anatomical position and transducer plane (longitudinal or transverse) [and type abbreviations of these directions onto main screen. Identify position as necessary throughout exam.]

C.   Examination Procedures

1.   Ask the participant the screening questions for the examination. Specifications for the Ultrasound Data Collection Form are attached.

2.   Place disposable glove on the hand that will operate the probe.

3.   Apply acoustic gel to the participant's upper right quadrant, and begin the scan. Survey the gallbladder area and identify the anatomical landmarks. Once the gallbladder is located, begin the VCR to record the examination.

4.   Scan longitudinally through the gallbladder to demonstrate a thorough examination of the gallbladder neck and fundus as well as a clear and sharp posterior gallbladder wall. Scanning may be performed subcostal and/or intercostally, whichever procedure provides the best view of the gallbladder.

5.   After the longitudinal scans are performed, stop the VCR tape, and change the transducer position annotation on the main screen. Start the VCR tape and begin scanning transversely through the gallbladder making clean sweeps from the fundus of the gallbladder to the neck.

6.   When satisfactory wall definition is obtained in the transverse view, freeze the image and measure the thickness of the anterior gallbladder wall. This is a single measurement and may be obtained in either the supine or left lateral decubitus (LLD) positions.

7.   When the supine screening is complete, stop the VCR tape, change the participant position annotations on the main screen. Ask the participant to turn onto the LLD position and start the VCR tape. Repeat steps 4 and 5.

8.   If views of the gallbladder are unobtainable in the supine position, the sonographer may move directly to the LLD scanning position. Note the omitted position in the appropriate section of the Ultrasound Data Collection Form.

4.2.2. Ultrasonography Reading Center (URC)

A maximum of 30 examinations will be recorded on each VCR tape. If, during an examination, a condition requiring rapid review is noted, that examination will be the last to appear on the tape and that tape will be mailed that day to the URC. All VCR tapes whether rapid review or not will be express mailed to the URC. Data collection forms will be completed and sent to the Data Coordinating Center on the same monthly schedule as other data. A URC radiologist will read each examination sequentially and complete a similar Ultrasound Data Collection Form and return it to the coordinating center with the VCR tape. Following data entry, tapes that contain examination recordings that resulted in disagreements in gallbladder findings will be returned to the Ultrasound Reading Center for adjudication using the same radiologist's form.

There are a few differences from the ultrasonography protocol of the NHANES.

1.   Automated data collection. Hard copy forms are used in NHANES only when the computer system is (uncommonly) down.

2.	A 5.0 MHZ probe as well as a 3.75 MHZ probe is used in NHANES. The 5.0 MHZ probe is used primarily to determine shadowing associated with wall irregularities and for better resolution in particularly thin persons. We have been advised by the NHANES 3 radiological consultant and the Accuson corporation that the 3.5 MHZ probe should be adequate for surveying the gallbladder. Nevertheless, if exam quality suffers, it may be necessary to purchase an additional probe.

3.	The data collection form has been simplified from 16 possible categories to 9. This was done after examining the results of the first 7,000 NHANES examinations and merging or eliminating codes that were rare and for which disagreement was common. All significant codes that can be used for analysis still match NHANES codes.

4.	For participants with gallstones, the radiologist is being asked to estimate the proportion of gallbladder volume that is being displaced by the gallstones. This measurement has been added because of the possible association of gallstone volume with risk of gallbladder cancer.

4.2.3. Interview data

Many risk factors pertinent to gallstones are being asked. A small amount of additional information would also be helpful:

1.	History of weight fluctuation - this may also be important for the cardiovascular component
2.	On a daily basis, when does the participant usually first and last eat a meal or snack?, - for overnight fasting period
3.	For women, in what year was her last child born? Did she breast feed that child and for how long? - gallstones are associated with recent pregnancy; metabolic effect of breast feeding, particularly increased energy usage and hormonal effects
4.	History of gallbladder surgery - if ask here, may not need to obtain at ultrasonography
5.	Current non-steroidal anti-inflammatory drug usage, besides aspirin - antinucleating effect in the gallbladder

4.2.4. Report of findings to participants

1.	At the examination, the ultrasonographer may, if asked, tell the participant the gallbladder findings. It must be made clear that the diagnosis is not definitive and that the participant will receive a letter with all their results.

2.	The letter to participants should state that the gallbladder was not seen, gallstones were seen, or the gallbladder had no stones. Interpretation would indicate that an absent gallbladder usually means the participant had gallbladder surgery and that gallstones usually do not require treatment unless they cause severe symptoms or complications. However, consideration of treatment must be made on an individual basis. In addition, conditions that require clinical follow-up, such as suspicious wall thickening suggestive of cancer, need to be noted, although not specified. The participant should be urged to have a follow-up evaluation. A physician referral form with the specific presumptive diagnoses can be completed and sent in accordance with the Strong Heart Study protocol.

## 4.3	Quality Control

4.3.1. Training

The gallbladder is a relatively easy organ to visualize by ultrasonography in a person who is not acutely ill. Nevertheless, training in the protocol is necessary even for an experienced ultrasonographer. Knowledge of hepatobiliary anatomy is necessary to identify landmarks and the gallbladder. Recognition of normal and abnormal findings in the gallbladder is critical, particularly wall irregularities and shadowing. Training requires demonstration of knowledge in these areas; it should be accomplished by short lectures on hepatobiliary anatomy and ultrasound principles, observation of gallbladder ultrasonography, and supervised gallbladder examinations. Training may be accomplished in a day for someone familiar with the equipment and ultrasound procedures.

4.3.2. Examination Sites

At the field clinics, the sonographers should have adequate opportunity to become comfortable with using the ultrasound equipment and VCR, filling out examination forms, and incorporating the ultrasonography into the necessary time constraints. Prior to formal data collection, approximately 20 ultrasounds should be performed at each site, recorded, and read at the URC. At least one and possibly two field visits for further training and to iron out problems with the examination may be necessary during the first year of examinations. Diagnostic agreement between the ultrasonographer and the URC and the radiologist's evaluations of the quality of the examination will guide the need for further training. It is expected that a kappa statistic of at least 0.8 will be maintained at each site between the readings of the ultrasonographer and radiologist.

## 4.4	Analysis

Analysis of the results of ultrasonography will be directed by the NIDDK program officer in accordance with the analysis and publications guidelines of the Strong Heart Study Manual of Operations. It is anticipated that manuscripts will be prepared in several areas:

1. Prevalence of gallstone disease among the 3 centers.
2. Risk factors for gallstone disease (could be several manuscripts).
3. Comparison of gallstone disease prevalence among the 3 centers with NHANES prevalence for Mexican-Americans, and non-Hispanic whites, and blacks.
4. Gallstone disease as a risk factor for coronary artery disease.
5. Secular trends in gallstone disease prevalence among Pima Indians (26-27 year time span).
6. If adequate follow-up occurs, association of gallstone disease with all cause mortality, heart disease morbidity and mortality, and site specific cancer mortality.

THE STRONG HEART STUDY II

Ultrasonographer Data Form

Strong Heart Study ID Number

Social Security Number

Date of Examination (mo/day/yr)

1. Ultrasonographer ID Number

2. Videocassette Number

3. Tape sequence Number

4. Have you ever been told that you had gallstones?
   1=Yes   2=No   9=Unknown

5. Have you ever had gallbladder surgery?
   1=Yes   2=No   9=Unknown

6. Including your last meal and any snacks, at what time did you last have anything to eat?

   Military Time:

   Day: 1 = Today   2=Yesterday

7. Time now (*please use military time*) (hh:mm)

8. Presence of surgical scar

   a.   Right upper quadrant   1=Yes   2=No

   b.   Epigastrium or periumbilical area   1=Yes   2=No

   c.   Laparoscopic scars   1=Yes   2=No

Ultrasonographic Findings

9. Portal vein at liver hilum on transverse scan?
    1=Yes   2=No   9=Unable to observe

    □

10. Liver margin on longitudinal scan?
    1=Yes   2=No   9=Unable to observe

    □

11. Intrahepatic right portal vein on longitudinal scan?
    1=Yes   2=No   9=Unable to observe

    □

12. Anterior gallbladder wall thickness in mm (on longitudinal scan)
    If unable to observe, fill in 99.

    □□.□

13. Can gallbladder be observed?
    1=Yes   2=No *(Skip to Question 20)*

    □

14. Were gallstones found?
    1=Yes (Echogenic clumps with shadowing in 2 views)
    2=No (Gallbladder visible, no echo clumps)
    3=No conclusion (Gallbladder clumps that shadow on only one view)

    □

15. If "YES," how many gallstones were there?
    1=Single   2=Multiple

    □

16. Measurement of largest echo clump (in mm)
    Fill in 0 if no clump was found, 99 if unable to observe.

    □□

17. Was gallbladder wall calcified? (Dense shadowing from gallbladder wall, exclusive of gallstones)
    1=Yes   2=No
    If "Yes," attach still image and send with video tape.

    □

18. Were cholesterol polyps found?
    (Echogenic clumps attached to gallbladder wall without shadowing that do not move)
    1=Yes   2=No

    □

19. Was gallbladder sludge observed?
    (Echogenic clumps without shadowing that move)
    1=Yes   2=No

    □

20   Were any other abnormal findings identified?   ☐
      1=Normal   2=Abnormal

      If "Abnormal," describe:_____

      _____

      _____

      _____

21.   Results of Examination: ☐
      1=Test done   2=Test incomplete   3=Test not done

22.   Reasons Test Incomplete or Not Done   ☐
      1=Ultrasound malfunction
      2=VCR malfunction
      3=Insufficient time
      4=Examinee refused or uncooperative
      5=Examinee medically excluded by staff for safety
      6=Examinee unable to physically cooperate
      7=Positive history of gallbladder surgery and visible right upper quadrant scar

23.   Comments?   ☐
      1=Yes   2=No

      If "Yes," Comments: _____

      _____

      _____

THE STRONG HEART STUDY II
The George Washington University Medical Center
Gallbladder Ultrasonography - Radiologist's Form

Strong Heart Study ID Number

Last Name

Date of Examination (mo/day/yr)

1.   Date of reading (mo/day/yr)

2.   1=First reading   2=Adjudication

3.   Radiologist ID number: 1=Dr. Hill   2=Others

Initial: _____
Initial

4.   Videocassette Number

5.   Tape sequence Number

Findings of gallbladder

6.   Adequacy of examination?
1=Adequate   2=Below standard   3=Inadequate

7.   Can gallbladder be observed?
1=Yes   2=No (*Skip to Question 16*)

8.   Were gallstones found?
1=Yes
2=No (Gallbladder visible, no echo clumps)
3=No conclusion (gallbladder clumps that shadow on only one view)

9.   If "YES," how many gallstones were there?
1=Single   2=Multiple

10.   Percentage of gallbladder filled with gallstones
1 = No gallstones   4 = > 50 %, but not filled
2 = <25 %   5 = Filled
3 = 25-50 %

11. Was gallbladder wall calcified? (Dense shadowing from gallbladder wall, exclusive of gallstones)
1=Yes   2=No □

12. Were cholesterol polyps found?
(Echogenic clumps attached to gallbladder wall without shadowing that do not move)
1=Yes   2=No □

13. Was gallbladder sludge observed?
(Echogenic clumps without shadowing that move)
1=Yes   2=No □

14. Other gallbladder abnormality

    a.   Gallbladder wall thickened (>3 mm)   1=Yes   2=No □

    b.   Contracted gallbladder   1=Yes   2=No □

    c.   Compatible with chronic cholecystitis.   1=Yes   2=No □
          However, underlying gallbladder cancer can not be excluded.

    d.   True polyp   1=Yes   2=No □

15. Certainty of gallbladder diagnoses:
1=Certain   2=Uncertain   □

16. Comments?
1=Yes   2=No   □

If "Yes," Comments: _____

_____

_____

Confirmed By: _____

5. Foot Examination

Background: Amputation and ulceration of the foot and lower extremities are important problems in the management of diabetes. Diabetes damages peripheral nerves. The nerve damage leads to poor sensation, loss of muscle tone, and repeated and sometimes unrecognized trauma to the foot. The trauma can be evident as ulceration, infection or fractures of the small bones of the foot.. Such fractures of the foot can cause deformities, and are known as "Charcot Joints".

It is clear that foot problems are often predictable in that they are highly correlated with loss of the ability to feel pressure, pain, vibration or to sense the position of the foot (proprioception). Thus, it is valuable to know whether patients have nerve damage. Such patients benefit from education about foot care, and they may need special footwear or therapy to prevent or delay foot and lower leg problems.

Figure 1. The Semmes-Weinstein Pressure Filament
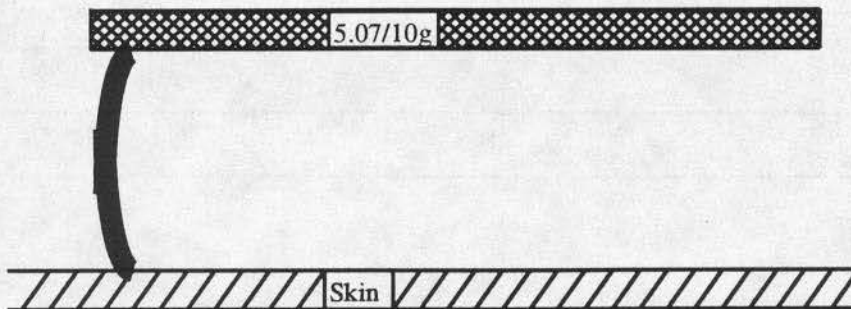
Figure 2. The filament should be placed against the skin at a 90°C angle. Apply pressure until the filament bends slightly, then ask the volunteer if they can feel the pressure.

The Semmes-Weinstein pressure filaments are a simple and reliable technique to detect the loss of pressure sensation in the foot. The Semmes-Weinstein pressure filaments is a simple, inexpensive device consisting of a plastic filament attached to a small handle.

Extensive studies of diabetic patients indicates that the loss of the ability to sense a pressure of 10 g on the great toe or 1st metatarsal head is highly correlated with ulceration. Ninety-five percent of norma individuals can detect the 10 g pressure.

Testing Pressure Sensation with the Semmes-Weinstein Filament:

1. Use the 10 g filament only.

2. The sites to be tested are indicated on the Diabetic Foot Screen Form. Examine the participant while he/she is in the *supine* position (lying down on their back)

3. Apply the Filament perpendicular to the skin's surface.

4. Apply sufficient force to cause the filament to bend. Keep it against the skin for about $1\frac{1}{2}$ seconds.

5. Randomize the selection of test sites and time between successive tests to reduce the potential for patient guessing.

6. Do not allow the filament to slide across the skin or make the repetitive contact at the test site.

7. Ask the patient to respond "yes" when the filament is felt and record the response on the Diabetic Foot Screen Form.

8. If the patient has a foot ulcer, apply the filament along the perimeter of the ulcer and NOT on an ulcer site, callous or necrotic scar.

9. Do not test parts of the foot that are covered by heavy bunions or callouses.

10. Express the foot exam results as a fraction such as "7/9." This means that 9 sites were tested and seven of them were felt.

THE STRONG HEART STUDY II

Diabetic Foot Screen

ID Number   [   ]

Name *(First, Last)* _____   IHS Chart Number   [| | | | |]

1. Is there a foot ulcer or a history of foot ulcer? (1=Yes 2=No)   ☐

2. Are the nails thick, too long or overgrown?   (1=Yes 2=No)   ☐

3. Is either foot numb?   (1=Yes 2=No)   ☐

4. <u>Label</u>: Sensory level with a "+" if the participant can feel the 10 gram filament and "-" if he/she cannot feel the 10 g filament. Test each site only once. Testing may not be accurate in areas where thick callous or bunion is present.

1=Positive   2=Negative
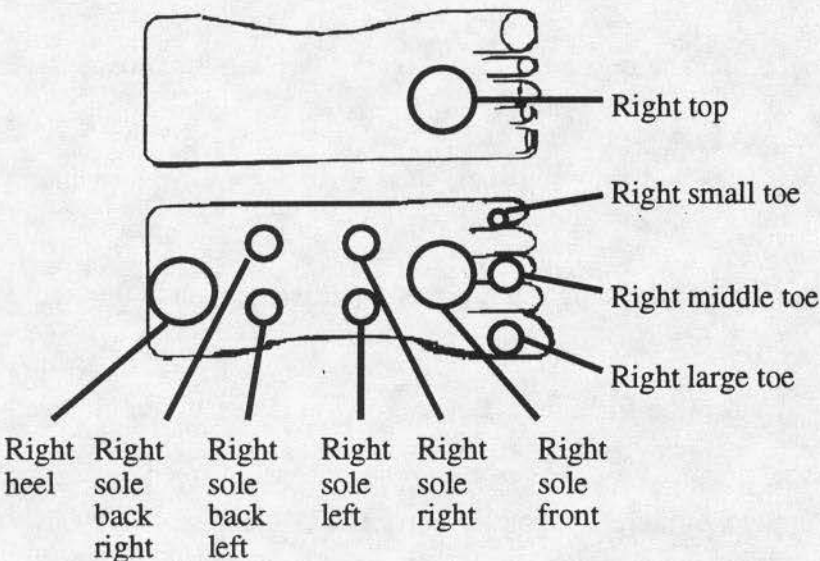
a. Right top   ☐

b. Right large toe   ☐

c. Right middle toe   ☐

d. Right small toe   ☐

e. Right sole front   ☐

f. Right sole right   ☐

g. Right sole left   ☐

h. Right sole back right   ☐

i. Right sole back left   ☐

j. Right heel   ☐

5. If the right foot has been amputated, conduct the exam on the left foot and make a note here:_____(approx date of amputation).

6. RESULTS: [| | / | |]

                                             Number of Number of
                                         correct answers sites tested

7. Examined by: _____   [| |]

8. Date examined   [| | / | | / | |]
                                                   mo   day   yr
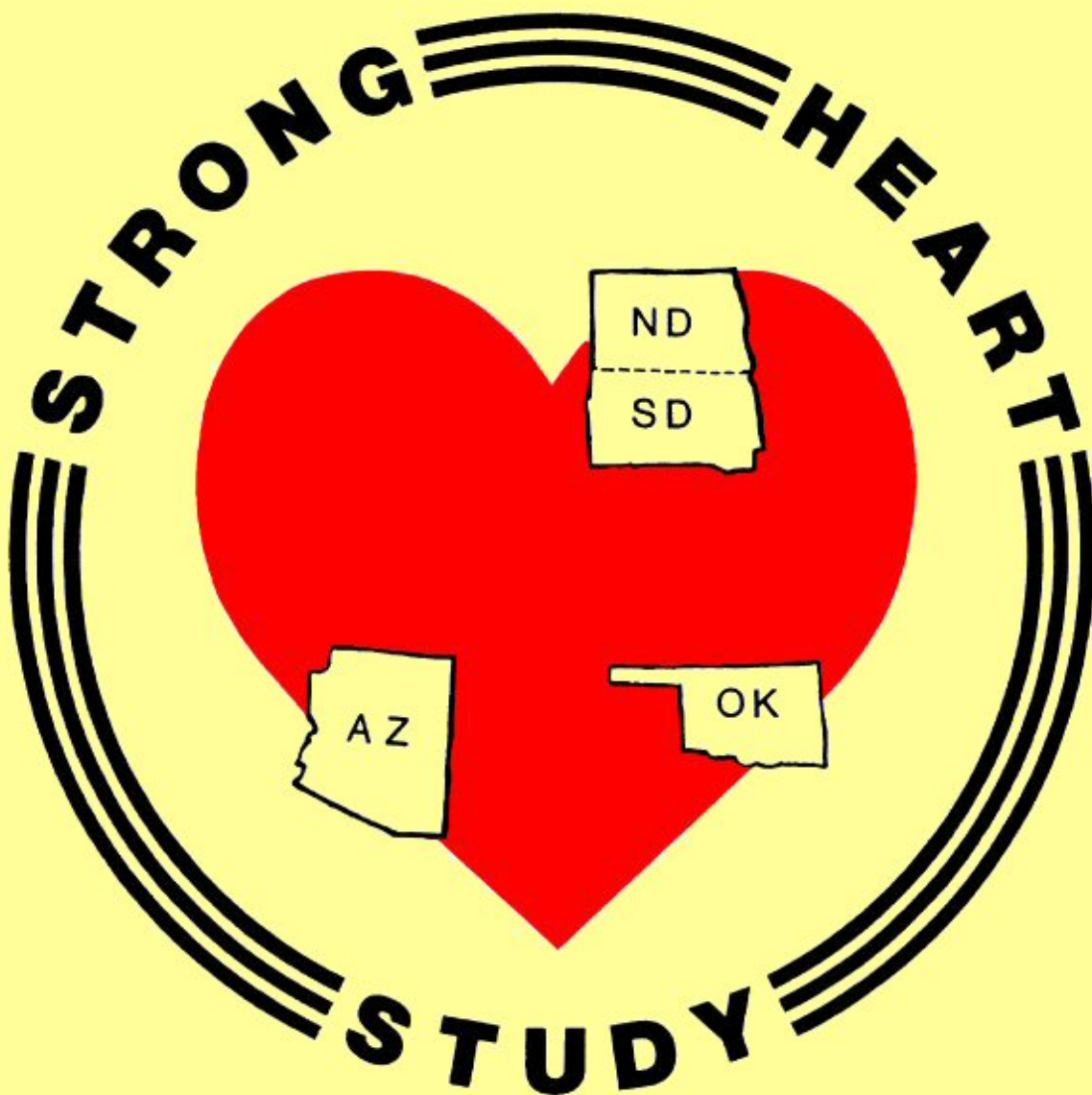
CARDIOVASCULAR DISEASE IN AMERICAN INDIANS
(PHASE II)

OPERATIONS MANUAL - VOLUME FIVE

DIETARY AND PSYCHOSOCIAL STUDIES

THE NATIONAL HEART, LUNG AND BLOOD INSTITUTE
OF THE NATIONAL INSTITUTES OF HEALTH

THE STRONG HEART STUDY

Cardiovascular Disease in American Indians
(Phase II)

Operational Manual

Volume Five

Dietary and Psychosocial Studies

July 1, 1993

MANUAL V

DIETARY AND PSYCHOSOCIAL STUDIES

TABLE OF CONTENTS

1. DIETARY INTERVIEW

1.1   Purpose of the dietary interview

       To obtain mean intakes of nutrients for males and females of different ages in the population.

1.2   Overview of the 24-hour recall

Introductions

- Introduce yourself.
- Communicate the importance of the subject's role in the Strong Heart Dietary study and the importance of receiving complete and accurate dietary information.
- Explain that the 24-hour recall will be part of the report on the average intake of all persons participating in the study, that it will be completely confidential and private.

Create an itemized list of foods consumed in a 24 hour period

- List all foods and beverages consumed during the **previous day**--a complete 24 hour period from midnight (12AM) to midnight (11:59)
- Specify eating times or meal names associated with these foods and beverages
- Ask if the way they ate was changed because of the fasting requirement

Fill in the list of foods and beverages with more detail

- Explain how to use the models to show the amount consumed
- Probe about each food and record as complete a description as possible
- Record the amount consumed
- Ask if something was added to the food and record the new food on a separate line.

Review the form with the participant again, giving time for any additions or alterations.

Ask about commonly forgotten foods

Complete the top section of the form.

Thank the participant for her/his time and repeat the value of all the information.

We strongly discourage interviewing the client in their home because all interviews should be carried out as similarly as possible. So, in the unlikely event you have to go to a client's home to do the dietary recall interview, **do not allow the client to get up and show you the food from the refrigerator or the cupboard.** Do not do anything differently than the interview done in the clinic.

General Probing Guidelines

- Ask questions in an open-ended way.

  For example, "What was the first thing you ate or drank yesterday?"

  Not "What did you have for breakfast?"

- Obtain additional information by probing.

  Relate eating to other activities, e.g. "Did you stop any place after work? Did you have anything to eat or drink there?"

- Ask non-leading questions, which do not expect a particular answer, to obtain specific detail.

  "Was that the same margarine as at breakfast?"

- Allow adequate time for the client to think about the answers.

- Be neutral in your responses to the information. Do not indicate approval or disapproval.

- Rephrase questions if the participant does not seem to understand the questions.

  "Did you have anything in your coffee?" explain as follows:
  "Did you put anything in your coffee before drinking it or was it plain coffee?"

- Stop probing when the client begins to be irritated or annoyed. We do not want the client to stop cooperating or to refuse the rest of the interview procedures.

1.3   General interviewing techniques

Before Beginning the Interview

Before beginning the interview, take some time to make sure you are thoroughly prepared. Review your manual and other materials given to you during training until you fully understand all aspects of your job. Practice doing the interview until you are comfortable with the procedure. This practice will help in building your confidence so that you can deal with any situation you may encounter when you begin interviewing. Your ability to work comfortably will help keep your respondents interested in the interview and will help your interviews go smoothly. Respondents quickly lose interest when the interviewer is constantly stopping, losing track of his/her place and stumbling in his/her efforts to ask questions or probe.

Check to make sure you have sufficient quantities of all necessary materials, and that your materials are organized in an orderly way. Materials that are to be handed to respondents should be easily accessible to avoid any awkward fumbling or searching.

The first thing a respondent notices about an interviewer is appearance. In general, an interviewer should aim at an appearance that is neat, suitable, and inconspicuous. Avoid extremes of any kind. Keep in mind that it is better to be a little underdressed than overdressed, and that, regardless of what clothes you wear, cleanliness and neatness are always very important.

1.3.1 Beginning the Interview

When you first make contact with a respondent, your initial task is to establish a friendly but professional relationship. Your own confident and professional manner will reassure the respondent and set a tone that will enable you to complete the interview in an efficient manner. Experience with past surveys has indicated that there are three main factors that determine whether a respondent will consent to be interviewed.

The Rapport Established Between the Interviewer and the Respondent

"Rapport" is the term used to describe the personal relationship between the interviewer and the respondent. Rapport provides the foundation for good interviewing. Your appearance, your introductory remarks, and the way you answer any questions the respondent may ask will strongly influence the rapport that develops between you and the respondent. What you say and how you say it should set the tone for the friendly, cooperative, but businesslike relationship that will continue to develop throughout the interview.

Whether the Respondent Sees the Survey as Being Important and Worthwhile

An interviewer must try to interest the respondent in the survey. Encourage the respondent to see the interview as an opportunity to express his/her views and to have those views taken seriously.

From the start, the respondent must be given certain basic information about the interview: what to expect during the interview, and what the purpose of the interview is. If asked, you may also explain how the information will be used, and the length of the interview.

Whether the Interviewer Can Respond Convincingly to the Respondent's Objections and Questions

Even respondents who are convinced of the importance of the study may, for a variety of reasons, be reluctant to grant a certain part of the interview. Your friendly manner, your introductory statements, and your success in answering the respondents' questions will help you sell both yourself and the survey to the respondents. Your effectiveness will be increased by your knowledge that your job is legitimate and important, and by your thorough understanding of what you are doing and how to do it.

Your own state of mind -- your conviction that the interview is important -- will strongly influence the respondent's cooperation. Your belief that the information you obtain will be significant and useful will help motivate the respondent to answer fully and accurately. Most people want to be heard and are happy that you have asked their opinions. Those who are reluctant to give specific information will often do so willingly, if they are convinced that good use will be made of it and that their privacy will be protected.

1.3.2 Administering the Interview

The interviewer's task is to collect accurate information. You must have a thorough understanding of the general principles for administering the interview and comprehend fully its confidential nature. The material in the following sections of this manual will acquaint you with the general principles and procedures to follow when collecting survey data.

Asking the Questions

The 24-hour recall is essentially an open-ended interview. Collecting accurate and reliable data requires that every respondent hears exactly the same questions read in exactly the same way. Even small changes in the way a question is asked can affect the way a respondent answers and, in the long run, affect the results when researchers combine the answers given by large numbers of respondents. The basic rules for asking survey questions, discussed below, are all designed to ensure uniformity in the way questions are asked of respondents.

Always Remain Neutral.

During the entire interview you must always maintain a completely neutral attitude. As an interviewer, you must never allow anything in your words or manner to express criticism, surprise, approval, or disapproval of the questions you ask or of the answers respondents give.

An important part of your role as an interviewer is to get the respondent actively involved in the interview, to lead him/her to talk comfortably and freely in response to your questions. While encouraging the respondents to talk freely, however, you must carefully avoid saying or doing anything to influence the content of the respondent's answers. No matter what topics you ask about, no matter how strongly you agree or disagree with the respondent's answers, and no matter how interesting, unusual or discouraging you might find those answers to be, you must always maintain the same neutral and professional stance during the interview. You are there to ask for and record the respondent's answers, not to influence or advise in any way.

At times, particularly if your respondent is talking freely, you may feel that he/she has already answered a question before you get to it. DO NOT SKIP OVER ANY SCHEDULED QUESTIONS, EVEN THOUGH YOU THINK THERE MAY BE SOME REPETITION. If a respondent becomes annoyed or says something like "I just told you that," you can acknowledge the repetition, but explain that you are required to ask all questions. You might say something like:

"I need to make sure that I have your full answer on that."

"I thought perhaps you might have more to say about that."

Sometimes it may be helpful to anticipate the respondent's reaction to the repetition by saying something like:

"You may already have mentioned this, but I need to make sure I have your answer recorded here."

"You may have told me about this before, but let me ask this questions to make sure I have the right answer."

To be a good interviewer, you must be comfortable with the questions you ask. If you feel uncomfortable with certain questions, it is likely that you will transmit something of that feeling to the respondent and influence the answers you receive. If you are uneasy with some questions, you should practice them repeatedly until you can ask them in a simple, straightforward, matter-of-fact way. Occasionally you will find a respondent who refuses to answer some questions, but usually you will find that as long as you can deal with all of your questions in the same relaxed and professional manner, your respondents will answer without hesitation.

MAINTAINING RAPPORT

You began your rapport-building process with your introduction, and it must be continued throughout the interview. Through accepting and understanding behavior and your interest in the respondent, you can create a friendly atmosphere in which the respondent can talk freely and fully.

Occasionally rapport may be broken during the interview for some reason such as the respondent feeling that a particular question is "too personal." If this happens, take time to reassure the respondent that he/she may speak freely without fear. This may be done be restating the confidential nature of the interview and the impersonal nature of the survey. If a respondent refuses to answer a question after you have reassured him or her of confidentiality, do not press the respondent -- enter a refusal response and the system will automatically skip to the next appropriate question. It is mandatory to attach a note to a refusal response.

Occasionally a question may lead a respondent to begin reminiscing or to relate a lengthy story that has little or no relevance to the survey. As an interviewer, your task is to discourage such irrelevant conversation and keep the discussion focused on the interview. In some ways, this requires that you subtly teach the respondent how to be a good respondent. If you maintain a businesslike attitude, acknowledge answers with neutral comments such as "I see," "OK," or a simple nod of your head, and tactfully interrupt rambling and irrelevant answers to bring the conversation back to the question you have asked, the respondent will soon learn how to be a good respondent and provide the kinds of answers you need. If you must interrupt a respondent, do it politely, taking care not to antagonize him/her. You might say something like:

"That sounds very interesting, but what I need to ask is..."

"I see what you mean, but let me repeat that last question..."

1.3.3 PROBING: WHAT IS PROBING AND WHY IS IT NECESSARY

Probing is the technique used by the interviewer to stimulate discussion and obtain more information. The quality of the interview depends a great deal on the interviewer's ability to probe meaningfully and successfully. We probe when a respondent's answer is not meaningful or is incomplete, i.e., when it does not adequately answer the question. There are a number of reasons respondents sometimes do not answer the question to our satisfaction.

In every day social conversation, people normally speak in vague and loose terms. It is understood that respondents will at first respond to our questions in a way which is not clear or specific. It is important to encourage the respondent to express himself/herself more concretely, in very specific terms.

Sometimes respondents will think that they are answering a question when all they are doing is simply repeating an answer which was already given, or simply repeating parts of a question. A respondent can talk a great deal and still be just repeating the question in different words.

Respondents will sometimes miss the point of the question. Many times they will give responses which seem to answer the question, but when you look further, are not to the point of the question and are therefore irrelevant. It is easy to be "taken in" by a respondent who is talkative and gives a lengthy and detailed response which, however, is quite beside the point. It is not the answer to the question asked. In most cases, a respondent gives an irrelevant response because he/she has missed an important word or phrase in the question.

Probing, therefore, has two major functions. First, probing motivates respondents to enlarge, clarify, or explain the reasons for their answers. Secondly, probing focuses the respondent's answer so that irrelevant and unnecessary information can be eliminated. All this must be done, however, without introducing bias or antagonizing the respondent.

Some examples of answers that for different reasons fail to answer the questions properly are given next. Because of the answers given, each requires probing.

EXAMPLES OF ANSWERS THAT REQUIRE PROBING

Question: How much plain water do you **usually** drink in a 24-hour period of time?

Answer: My doctors says I should drink more water.

Probing Methods Should be Neutral

It is very important to always use neutral probes. By this we mean you should not imply to the respondent that you expect a specific answer or that you are dissatisfied with an answer.

Remember the reason for probing is to motivate the respondent to respond more fully or to focus the answer, without introducing bias. The potential for bias is great in the use of probes. Under the pressure of the interviewing situation, the interviewer may quite unintentionally imply that some answers are more acceptable than others or may hint that a respondent might want to consider this or include that in giving responses. You must be careful not to do this.

The following example consists of a response from the client, and two possible problems. The first of these probes is neutral, the other is not.

Example:

Client: I had a cup of coffee at 9:00 a.m.

Neutral Probe: Did you add anything to your coffee?

Non-neutral Probe: So you probably added cream and sugar?

The non-neutral probe suggests a specific answer to the respondent and thus leads the respondent toward that answer, rather than leaving the range of possible responses completely open for the respondent to specify.

1.3.4 Kinds of Probes

There are several different neutral probes which appear as part of a normal conversation that can be used to stimulate a fuller, clearer response.

1. **AN EXPRESSION OF INTEREST AND UNDERSTANDING.** By saying such things as "un-huh" or "I see" or "yes," the interviewer indicates that the response has been heard, that it is interesting and that more is expected.

2. **AN EXPECTANT PAUSE.** The simplest way to convey to a respondent that you know he/she has begun to answer the question, but has more to say, is to be silent. The pause -- often accompanied by an expectant look or a nod of the head -- allows the respondent time to gather his or her thoughts. Eye contact is important here.

3. **REPEAT THE QUESTION.** When the respondent does not seem to

understand the question, or misinterprets it, or seems unable to decide, or strays from the subject, it is often useful to repeat the question. Many respondents, when hearing the question for the second time, realize what kind of answer is needed.

4. **REPEATING THE RESPONDENT'S REPLY.** Simply repeating what the respondent has said is often an excellent probe. Hearing the response just given often stimulates the respondent to further thought.

5. **A NEUTRAL QUESTION OR COMMENT.** Neutral questions or comments are often used to obtain clearer and fuller responses. The following are some suggestions for probing questions that may help explore many types of insufficient answers.

PROBES TO CLARIFY:

"What do you mean exactly?"

"What do you mean by...?"

"Could you please explain that a little? I don't think I quite understand."

PROBES FOR SPECIFICITY:

"Could you be more specific about that?"

"Tell me more about that."

PROBES FOR RELEVANCE:

"I see. Well, let me ask you again...(REPEAT EXACT QUESTION)."

PROBES FOR COMPLETENESS:

"What else?"

"What else can you think of?"

I Don't Know (DK) Response

The "I don't know" answer can mean a number of things. For instance,

- The respondent doesn't understand the question and says DK to avoid saying he/she doesn't understand;

- The respondent is thinking the question over, and says DK to fill the silence and give himself/herself time to think;

Try to decide which of the above may be the case. Don't be in too big a rush to settle for a "don't know" reply. If you sit quietly -- but expectantly -- your respondent will usually think of something. Silence and waiting are frequently your best probes for a "don't know" reply.

Always try at least once to obtain a reply to a "don't know" response, before accepting it as the final answer. But be careful not to antagonize respondents or force an answer. If they say again that they "don't know," proceed to the next appropriate question after coding the DK reply.

Additional Guidelines for Probing

The following rules and examples provide further guidance to help you select problems that will not bias respondent's answers.

1. Don't ask "Do you mean _____ or _____?" Such a probe suggests only one or two possible answers, when the respondent may actually be thinking about other possibilities. Do not use probes for clarity and specificity when a respondent's answers are unclear.

Example

Question: Did you put anything on your grapefruit?

Answer: Yes, Sweetener

Neutral Probe: Could you be more specific? What type of sweetener?

Non-neutral Probe: You mean it was Equal or Sweet 'n Low?

Example

Question: What did you eat at that time?

Answer: I had eggs and juice

Neutral Probe: Did you eat or drink anything else at that time?

Non-neutral Probe: So you had breakfast -- you probably had coffee, too then?

When to Stop Probing

When you have obtained all necessary information about the respondent and when you have encouraged the respondent to clarify the meaning of his/her own words so that you (and we) know exactly what he/she had in mind -- only then do you have a complete answer and only then should you stop probing. However, if at any time the respondent becomes irritated or annoyed, discontinue probing. We do not want the respondent to refuse the rest of the interview.

1.3.5 Ending the Interview

All people who give their time for an interview are entitled to courteous and tactful treatment. Try to leave respondents with the impression that they have taken part in an interesting and worthwhile experience -- one they would be willing to repeat.

After all questions have been asked, indicate your appreciation to respondents by thanking them; also mention that their contribution has been most helpful in providing important information to the study. Remember that the respondent is familiar with your task from the discussion at the beginning of the interview, so don't spend too much time going over the same information. Spend a few minutes answering any additional questions your respondent may have; then close the interview.

1.3.6 Editing

After you have completed the interview with a respondent, you are to edit or check your work. You should try to complete the edit before the client leaves. Once the client leaves, data that were not collected or data that needed to be corrected are lost forever.

Although editing is not difficult, it is an important part of an interviewer's job. The main purposes of editing are:

1. **TO REVIEW ALL SECTIONS OF INTERVIEW** -- Review each section to assure all information is complete and accurate.

2. **TO LEARN FROM MISTAKES SO THEY ARE NOT REPEATED** -- There is an educational value in editing. Many interviewers feel that the interviewing procedures become more meaningful after they have conducted the first few interviews. Editing will improve the quality of your interviews for the remainder of the study, as well as catch errors. Editing, therefore, is part of the learning process for a survey.

3. **TO WRITE OUT ABBREVIATIONS** -- The clerical aspect of editing is an

obvious one. This includes checking to make sure any abbreviations that are not commonly understood are clarified, and to assure that notes and any other comments are presented for easy comprehension.

4.   TO ADD YOUR COMMENTS WHICH MIGHT HELP TO UNDERSTAND A RESPONSE OR AN INTERVIEW AS A WHOLE --
Add notes concerning the respondent, the interviewing situation, or anything else that you feel might help in the correct interpretation of the interview.

1.4   DETAILED FORMAT FOR THE 24-HOUR RECALL

Before beginning the dietary recall, record:

1.   The subject's name and ID number (social security or strong heart) in participant ID section.
2.   Your ID number and initials in interviewer ID section.
3.   The date of the visit, the visit number, and the intake day (i.e. the day being recalled).
     subject's date of birth, sex - M / F.

1.4.1 Introductions

Introduce yourself and communicate the importance of the subject's role in the Strong Heart Dietary study and the importance of receiving complete and accurate dietary information. Explain that the data you will be collecting will be part of a report on the average intake of all persons participating in the study and that it will be completely confidential and private.

For example:

> "Hello, my name is (   ). I work with The Strong Heart Dietary Study and we are collecting information on what people in your age group eat and drink. We are going to look at this information to find out if some of the foods you eat or the way they are cooked lead to heart disease." Everything you say here will be kept confidential."

1.4.2. Create an itemized list of foods and beverages consumed

The client must understand that you are interested in recording everything eaten or drunk during the 24 hours of the previous day, from midnight to midnight. It is important to define the day and time exactly, such as, "We'll be talking about the period from 12 midnight Tuesday to 12 midnight last night. If the client were to report eating an item at midnight the day before and the night before the exact time frame is 12:00 am to 11:59 pm.

Explain to the client that during the first step of simply listing the foods and beverages consumed, the client must tell you the approximate time the foods and beverages were eaten. Have the respondent categorize the foods and beverages by using a time frame, e.g., "At 8:00 am, I had coffee and eggs. At 10:00 am, I had a doughnut."

Inform the client that you will be asking for more detail about these foods later.

Emphasize that the client is to tell you everything eaten or drunk, including snacks, coffee breaks and alcoholic beverages, at home or away from home. Include tap and bottled water, Perrier, mineral water, herbal tea, alcoholic beverages and pop or soda. Do not include chewing gum, or chewing tobacco (snuff or chew), or any fiber supplements.

For example,

> "What we want to do first is to make a list of all the foods and beverages that you have had in a 24 hour period of time, a complete day. This includes alcoholic beverages, pop or soda, tap water, mineral water like Perrier, herbal teas, Indian teas and tap or spring water. Remember to include all snacks."

> "Today is (day of the week)... I'd like you to tell me everything you ate or drank all day (yesterday)... from midnight (previous night) until midnight (yesterday). This means if you went to bed (previous night) after midnight, and you ate or drank something, you would start there. If you were asleep at that time, start with the first thing you ate or drank after you woke up yesterday."

> "I would also like you to tell me what time you ate. For example, at 8:00 am I had this, at 10:00 I had that." We'll make a very general list at first, then we'll go back and fill it in with more detail."

> You can start with the first food whenever you're ready."

As the participant tells you what he/she ate, record each food or beverage on a separate line. Record enough detail so that you can remember what they ate and ask for more detail later. If they give you more detail than you need, record this but remember to check it during the second part of the interview. Don't worry about the order of the foods.

For example, if the client says coffee, eggs, and toast, enter coffee on the first line, eggs on the second line and toast on the third line. When you go back through the 24 hour recall form for the second part of the interview, you will ask what he/she added to their coffee. If sugar and cream are mentioned, record sugar and cream on separate lines. Put

the line number of the coffee in parentheses next to the sugar and cream so that the data entry person know that these items were in the coffee.

Try not to interrupt the respondent--the only exception is if they forget to give you the time consumed (or a meal name). If the respondent is unable to recall what they ate, use non-leading probes, such as:

1) "What was the next thing you had?"
2) "What else did you have at that time?"
3) "Perhaps it will help you to think about what you did yesterday."

Don't mention a meal name or ask about foods they usually eat: i.e. "What did you have for breakfast?" or "Do you usually have a cup of coffee first?"

Print clearly in ink. Use additional lines freely. If you or the subject makes an error, draw a line through the entry, and rewrite it either on another line, with the appropriate time and place, or at the bottom of the form, with reference to the original line number. Mark continuation pages as required.

1.4.3. Ask about fasting

After the foods are listed, you need to assess if the participant changed their usual eating habits because of the fasting requirement. For example, "Did you change the way you ate last night between the hours of ... and ... because of the fasting requirement?" If they did, ask: "How did you change the way you ate?"

If the amount of food or the time eaten had changed, continue with the interview.

If food or beverages were not eaten because of the requirement, ask what the participant had eaten during that time period the previous evening (two evenings before the interview). Record these foods but **note** with an asterisk (*) that they were from the previous evening.

Fill in the list with more detail

For every food, you need to complete the following information: time eaten, and the place food was prepared (1=home, 2=restaurant). In addition, you will be asking the amount consumed and for a complete description of the food (including if salt was added in preparation or at the table, fat was added in preparation, and type of fat used in preparation.

You can introduce this next step by saying:

"We are now going to fill in this list with more information about the foods

you ate."

1.4.4. Amount of food consumed

Introduce the use of food models and measures. For example:

> "I need to know the amount of the foods and beverages that you ate. As we go through the list of foods, you can use any of these models (point to them) to show me how much of each food you ate. If you use the spoons, please tell me the amount in terms of level spoonfuls."

It is good practice for the participant to show you using one of the models even if they explicitly state the amount, i.e. the participant responds verbally with the number of cups or ounces. For example, if a respondent says: "It was an 8 oz glass", your response should be: "Please show me with one of the models."

After they've shown you, **always** ask: "How many did you eat?" Also ask if they ate or drank the whole portion. For example, "How full was the glass? How much did you drink?"

We don't record foods in amounts less than one tablespoon unless it is a fat, salty food, sugar, artificial sugar or other food that is a nutrient or calories dense source. **No matter what quantity is reported, artificial sweeteners must always be recorded**.

1. Fats include: margarine, oil, butter, nuts, coconut, salad dressing, avocado, cream cheese and other cheeses, non-dairy creamer and cream

2. Salty items include: soy sauce, teriyaki sauce, tamari, mustard, catsup, pickles, bacon bits, olives, anchovies and caviar.

3. Sugars include: sugar, honey, jelly, jam, corn syrup, fructose, pancake syrup and chocolate syrup.

4. Others: protein supplement, oat or wheat bran, wheat germ, vegetables high in Vitamin A/C.

If the respondent is having trouble with a particular model or the model seems inappropriate, suggest that they use a different model. For example, they pick up a teaspoon but say it was heaping and can't say how many level teaspoons it was.

Have the participant point to lines on the models (i.e. 4 oz, 6 oz, 8 oz.). Record both sizes if subject states his serving was between 2 sizes, e.g. 1/2 to 3/4 cup.

<u>Food-specific guidelines</u>

1) Try to get beverages without ice. If this is impossible, note that the amount was with ice.

2) Try to get the amount of meat without the bone. If you cannot, have the participant estimate the size of the bone.

3) If you record the brand for packaged foods (eg. candy bars, soda/pop, cookies, crackers, pre-sliced cheeses or luncheon meats), the participant does not need to use a food model but try to be aware of different serving size options that are commonly available and record this (eg. mini- versus regular-size candy bars).

4) The form is important for some foods, i.e. chopped, diced, melted, solid, ground, or shredded (eg. cheeses or meats). Some of these are noted in the probing guidelines in Appendix I.

5) Thickness is also important for some foods (eg. fry breads, pieces of cake/pie, homemade tortillas)

1.4.5. Completing the food description

After filling in the amount of a food consumed, you need to get as complete a description of that food as possible. Use open-ended questions that are non-leading but specific to the kind of food. Appendix I contains questions you should ask about each kind of food. Follow these probing guidelines for each food or food group. If you have to mention specific options, try to give more than 2 options and always say "or some other type". For example, "At 6am you drank coffee, was the coffee brewed from ground, instant, from a vending machine, or some other type of coffee?"

Fill in the columns about salt added in preparation or at the table and fat added in preparation. Note the type of fat and/or the brand name used in preparation in the description; **the type of fat used in preparation or added to foods is very important to the goals of this study**. If the client ate in a restaurant or at someone else's home, ask if they thought that salt was added and as a last resort "if the food tasted like salt was added". If they say "Yes" then mark on the form that salt was added in preparation.

Obtain brand names of commercially-prepared foods, however, be careful because some respondents may use a brand name instead of a generic name to refer to a food. For example, Coke instead of cola or koolaid versus some powdered mix. Probe: "Was is actually Coke or was it another brand of soda or pop?"

Record the names of restaurants and fast food establishments where the food was prepared.

If a subject is unsure of how the food was prepared or the ingredients, record what he called the food, in "quotation marks" and note as much as possible even if it seems too vague. For example, general categories of foods (eg. a vegetable), color, and shape.

Appendix II lists certain foods for which you should ask the participant to tell you the ingredients. These are foods that have highly variable ingredients and for which a participant should be able to list the component parts. Sometimes obvious foods are forgotten, eg. they said they ate a sandwich and you've asked them for more detail but they forget the bread. It's OK to ask: "Was that eaten plain, on bread, a roll or with something else?"

Always ask: "Did you add anything to your (food)?" even if it seems unlikely. Indicate foods eaten together by putting brackets around them.

Examples of food-specific probes:

1) "How was the gravy made?"
2) "Did you eat any of the vegetables that you cooked with the meat?"
3) "How much fat was left after you trimmed the meat?"
4) "Can you describe how your eggs were prepared?"

1.4.6. Review the foods

After you have filled in the description and amount of each food, read the list of foods and amounts to the participant quickly to ensure accuracy and completeness. Tell the participant what you are going to do before you start and ask them to stop you at any time if they remember something else or if something needs to be corrected.

Once you have finished, read through the list of "commonly forgotten foods". Add any foods that they have omitted using the same instructions as for other foods.

RECALL REVIEW

Since the information that you have given me will make an important contribution to this health and nutrition study, I would like to make sure that it is as complete as possible. On this card are some foods and beverages which are often forgotten.

Fruits, Chips, Candy, Nuts, Cheese

Coffee, Tea, Soft Drinks, Juice, Water

Beer, Wine, Cocktails, or Any Other Alcoholic Beverage

Crackers, Breads

Can you think of anything else that you ate or drank yesterday that you haven't mentioned?

Complete the remaining questions on the form

Record your opinion of the reliability of the information. An explanation must be given for any recall not coded reliable. This code only refers to the quality of the 24 hour recall.

1.4.7. Reliability

Reliable: You feel that the respondent made a sincere effort to answer the questions, and that the information given is probably correct. Include recalls in which the respondent does not know certain ingredients in a recipe, such as in a casserole, or whether fat was used in preparation. Classify the recall as reliable even if the majority of food amounts are unknown.

If you perceive the foods reported by the client as accurate, yet he/she says he/she cannot remember his midnight snack, still code this as a reliable recall. In the latter situation, the client has made a sincere effort to report all foods, but just cannot remember his/her snack; the information is reliable.

Unreliable: You feel the respondent was not able to give information that you think is correct. Include, for example, an elderly client who cannot seem to understand the instructions or remember what was eaten, or a client who was drunk. Before coding an interview as unreliable, make sure you have exhausted all sources of possible proxies for the client. An elderly client may have been accompanied to the center by a daughter or son who cares for and feeds the client. Do not code unreliable in cases where you do not believe the client, such as an obese person who reports very little intake.

Refusal: The client refuses to do the interview before you are able to obtain any information.

Not Interviewed: Due to time constraints, there will be occasions when the dietary interviewers are unable to complete interviews on those scheduled for a specific session. A mandatory note is required to explain the situation. Since clients with medical problems may return in the future once their problems have been treated, attach a note "sent home due to medical problems; may be rescheduled".

In general, if a client recalled food items for the previous day and you were able to record them even though the client had some difficulty with detailed descriptions or amounts, this would still be a RELIABLE interview. An example of an UNRELIABLE

interview is a situation where an elderly client reported an entire day's recall and during the Recall Review said those were not the foods he actually had. In this case the client was confused and unlikely able to accurately give a day's recall, especially in the time you would have. In a case like this, record the interview as UNRELIABLE; do not take the additional time to try to start over. If this type of situation happens and after 15-20 minutes you can tell that the client cannot provide a reliable interview, end the interview, thank the client, and attach a note explaining the situation. Check to see if a proxy is available to give the recall for the client.

Ask the participant if "the amount of food they consumed yesterday was typical, considerably less than usual, or considerably more than usual".

Read the question about vitamin or mineral supplement use and if they took one the previous day, list these as a food item. Remember to ask for brand names of vitamins, eg. One A Day Vitamins, and how many they took and dose if possible.

Thank the participant!

Thank the participant for her/his time and repeat the value of the information they have given you.

APPENDIX 1: RECALL REVIEW

Since the information that you have given me will make an important contribution to this health and nutrition study, I would like to make sure that it is as complete as possible. On this card are some foods and beverages which are often forgotten.

Fruits, Chips, Candy, Nuts, Cheese

Coffee, Tea, Soft Drinks, Juice, Water

Beer, Wine, Cocktails, or Any Other Alcoholic Beverage

Crackers, Breads

Can you think of anything else that you ate or drank yesterday that you haven't mentioned?

APPENDIX 2

ASK PARTICIPANTS TO LIST INGREDIENTS FOR THESE FOODS

SANDWICHES: Most clients can give some information about a sandwich. The client should be able to describe a sandwich purchased in restaurant. He/she should be able to tell you if the bread was brown or white, hot dog, hamburgers, chopped or shredded meat or poultry mixed with barbecue sauce on a bun. He/she should be able to tell you if there was lettuce and tomato on it. Certain sandwiches are fairly standard, and if not prepared by the client, are difficult to specify. You do not need to break down sandwiches purchased at national fast food chains into their component parts.

TOSSED SALADS: It is preferable to have a client list and quantify the items in a tossed salad. Remember to apply the small amount recording rule to avoid entering and specifying items which are not necessary due to the small quantity consumed. Should the client know the components of the salad, you can determine the amount based upon whether the client can provide you with information as to the proportion of the ingredients in the salad, for example, "2/3 lettuce and 1/3 other vegetables".

If the client reports a salad but cannot break it down into its individual components, get as much of a description as possible (eg. iceberg versus leaf lettuce, with or without tomatoes).

MIXED DISHES AND SOUPS: This usually refers to recipes that are mixed together and served together as a dish rather than assembled at the table. Include in this category such items as pizza, spaghetti with sauce, meat loaf, beef stroganoff with noodles, beef and broccoli stir fry, quiche, pot pies and casseroles. In general, it is best not to attempt to have the client break down recipes that may have many ingredients into their component parts.

Macaroni and cheese: ask if it was a box mix or if it was made from scratch. If it was made from scratch ask the kind of milk (whole, low fat or skim) and if butter or margarine was added. Remember to ask for brand names of butter and margarine.

TACOS: Tacos can be highly variable. Ask about the kind of tortilla (corn or flour), whether the tortilla was fried or warmed without fat (on a griddle), and what was contained in the topping.

VEGETABLES: Use the color of the vegetable as the main indicator of its nutritional importance. If the vegetable is green, orange or red, the vegetable is worth noting. Do not count small amounts of onions, mushrooms or celery. Do count broccoli, green peppers, orange squash, carrots and tomatoes. Remember that the small amounts recording rule still applies for those vegetables low in Vitamin A and/or C.

APPENDIX 3
PROBES ABOUT TRADITIONAL FOODS

Berry pudding, may be called "wojapi" in the Northern Plains:

- wild berries or canned berries used
- the type of berry eg. blueberries, blackberries
- corn starch or flour was used to thicken it
- sugar or an artificial sweetener was added to it.

Burrito
- type of filling
- sauce added?

Cheese crisp
- type of cheese
- with chili peppers?

Chili
- stew or sauce?
- if stew, with meat and/or beans? red or green chili?

Chili peppers
- the variety or at least the color, size, and shape.

Cholla bud stew
- type of fat used

Chorizo sausage and egg
- enter egg separately and record type of fat used

Corn, squash, and cheese
- type of fat used
- type of cheese

Enchilada
- type of filling
- type of sauce

Fry bread
- type of fat used in frying
- diameter and height

Guyvsa
- type of fat added if any

Lazy bread
- type of fat

Menudo
- get recipe or at least ingredients (eg. white corn, beef feet)

Posole
- get recipe or at least ingredients (eg. wheat and beans)

Pinole
- milk added instead of water?

Red chili stew
- type of fat used

Salsa or chili (sauce not a stew):
- red or green salsa
- commercially-prepared or homemade
- if homemade, was fat used in the preparation and the type of fat used

Skillet bread
- type of fat used
- size (diameter)

Soup with turnips:
- wild (timpsila) or store bought turnips

Taco
- fry bread or tortilla (corn or flour)
- if tortilla, fried or warmed without fat?
- ingredients in topping or filling

Tamale
- type of filling
- type of sauce
- dimensions including height

Tepary beans
- type of fat used if any

<u>Tortillas</u>:
-      corn or flour tortillas
-      fried or warmed without using fat

<u>Tortilla soup</u>
-      get recipe or at least ingredients

<u>Wild spinach</u>
-      type of fat used if any
-      additions (eg. chili peppers, tomatoes)

1.   **BEEF**

-      Was it a steak, roast or ground? If ground, was it regular, lean or extra lean?

-      What cut?

-      Was fat trimmed or not trimmed?

-      How was it prepared?

-      Was anything added before, during or after cooking, e.g., marinade or coating? If marinated, was it a soy sauce or non-soy sauce mixture? If coated, was coating eaten?

-      Fat and salt added in preparation?

2.   **BEVERAGES**

    A .   **Beer**

-      What type (i.e., regular, light, low alcohol, malt or nonalcoholic)?

    B .   **Cocktails, liquor, and liqueurs**

-      Name of drink or type of liquor or liqueur and what was added to it?

-      Accompaniments (i.e., fruit, olive, cherry)?

-      With or without ice, or frozen where applicable (i.e., daiquiri).

C. Coffee

- Type (i.e., regular, espresso, specialty coffees, coffee substitutes)? **Get** brand name for coffee substitutes, and regular or low calorie for flavored coffees.

- Regular or decaffeinated?

- Ground, instant, liquid, or vending machine?

- Accompaniments (i.e., milk, cream, sugar, artificial sweetener)?

- Liquid or dry amount?

- Plain or flavored?

D. Juices

- What kind?

- Real juice or a juice-flavored drink? Get brand name, and type of sweetener for juice-flavored drinks. If brand name unknown, get flavor, regular or low calorie; or whether fortified with vitamin C.

- Fresh, frozen, or ready-to-drink?

- Sweetened or unsweetened?

- Regular or no salt (where applicable, such as tomato or V-8 juice)?

- With or without ice?

E. Soda

- Brand name (if known)?

- Flavor?

- Regular or diet? If diet, was it sweetened with aspartame or saccharin?

- With or without caffeine?

- With or without ice?

F. Tea

- Brewed, herbal (flavor), instant, or ready-to-drink? If sugar-free instant, get brand name.

- Regular or decaffeinated?

- Unsweetened or presweetened with sugar or artificial sweetener? If artificial sweetener, was it aspartame, or saccharin?

- Accompaniment (i.e., lemon, sugar, or cream added)?

- With or without ice?

G. Water

- Quinine, tonic, or mineral? If quinine or tonic, was it regular or diet?

H. Wine

- Name?

- If name is unknown, red, white, rose, or sparkling; table or dessert; regular or homemade?

- Plain or mixed (i.e., spritzer, cooler)?

I. Cocoa

- Brand name? Regular, sugar-free, or low calorie?

- Recipe or dry mix? If recipe, what percent milk was used? If dry mix, regular or sugar-free? Was water or milk added? If milk added, what percent fat?

- Any additions (i.e., marshmallows, whipped topping)?

3. **BREAD**

A. Bread

- Kind (i.e., white, wheat, rye, etc.)?

- Homemade or commercial?

- Regular or diet? Low sodium? If diet, with or without added fiber?

- Toasted or untoasted?

- Accompaniments?

B. **Rolls or buns, bagels, English muffin and biscuits**

- Kind?

- Baked commercially, or from refrigerated dough, mix, or recipe? If recipe, ask type of fat for biscuits.

- Accompaniments?

C. **Coffee cake**

- Yeast or quick bread?

- Type of fat in preparation of cake (and topping)?

- Fruits, nuts, fillings, frosting, glaze, and/or streusel topping?

D. **Cornbread**

- Regular or stuffing?

- Type of fat in preparation?

- Accompaniments?

E. **Danish and sweet rolls**

- Fruit, nuts, filling, frosted? If frosted, type of fat in frosting.

- Type of fat used in preparation?

- Accompaniments?

F. **Donuts**

- Yeast, cake, or filled? If filled, with cream/custard of fruit/jelly?

- Plain, frosted, glazed, or powdered sugar? If frosted, what flavor?

- With or without coconut?

G. French Toast

- Fat used in preparation?

- Accompaniments?

H. Fruit Breads

- Type of fat used in preparation?

- Nuts?

- Accompaniments?

I. Muffins

- Kind?

- Prepared from mix, commercial, or scratch?

- Type of fat used in preparation?

- Fruit or nuts?

- Accompaniments?

J. Pancakes and Waffles

- Kind?

- Type of fat used in preparation?

- Fruits, nuts?

- Accompaniments?

K. Tortillas

-       Flour (white or whole wheat) or corn?

-       Plain or fried?

-       Accompaniments?

4.   **CAKES**

-       Kind (e.g., yellow, devil's food, white, pound, etc.)?

-       Mix, ready-to-eat, or recipe? If recipe, type of fat used in preparation. If mix, oil added in preparation?

-       Kind of frosting, glaze, or topping? If made from recipe or mix, type of fat used?

5.   **CANDY**

-       Brand name?

-       If brand if unknown, get complete description (kind, coating, filling, nuts). If contains chocolate, what kind? Is it a candy bar or individual pieces?

-       Size of candy bar (i.e., regular, miniature, etc.)

6.   **CEREAL**

A.   Cold   (ready-to-eat)

-       Brand name (or kind if brand unknown)?

-       Plain or presweetened?

-       If granola, brand name or was it a recipe? If recipe, type of fat used in preparation. Coconuts or nuts added?

-       Accompaniments (i.e., milk, sweetened, fruit)?

B.   Hot

-       Kind (includes some brand names like Wheatena or Maypo)?

- Flavored?

- Regular, quick cooking, or instant?

- Cooked with water or milk? If milk, what percent fat?

- Cooked with or without salt?

- Accompaniments (i.e., milk, sweetener, fats)?

7. CHEESE

A. Cheese

- Brand name?

- Type (i.e., processed, imitation, natural, cheese food, cheese spread; low sodium, low fat, low cholesterol)?

- Name (i.e., cheddar, Swiss, mozzarella, etc.)?

- Form (i.e., sliced, shredded, brick)?

B. Cottage Cheese

- Percent of fat (creamed or uncreamed)?

- Low Sodium? Low Fat?

- Additions (i.e., fruit, vegetables, sweetener)?

8. COMMERCIAL FOOD ENTREES

- Brand names?

- Description of product

9. CONDIMENTS

- Kind (i.e., catsup, mustard, pickles, etc.)? If mustard, regular, Chinese, or horseradish?

- Low calorie or low sodium?

-       If homemade BBQ sauce, specify fat.

10.   COOKIES AND BARS

-       Kind and brand name?

-       If brand unknown, commercial, mix/dough, bakery, or recipe? If recipe, type of fat used in preparation?

-       Nuts, chips, fillings, raisins, frosting or icing?

-       Dietetic?

11.   CRACKERS

-       Brand name (or kind, if brand name unknown)?

-       Regular, low sodium, or unsalted, where applicable.

-       Accompaniments (i.e., spread, cheese, deli meat)?

12.   CREAM/CREAMER

-       Kind (i.e., real, imitation)?

-       For real cream, ask type (i.e., heavy, light, half and half).

-       For non-dairy creamers, get brand name. If unknown, was it liquid/frozen or powdered?

-       For whipped cream, get brand name or type (i.e., aerosol, frozen, powder, or recipe). If recipe, get type of cream used. Sweetened or unsweetened? If aerosol or frozen, dairy or non-dairy. If powder, regular or low calorie?

-       For sour cream, get type (i.e., regular, half and half, or non-dairy substitute).

13.   DESSERTS

A.   Pudding/Custard

-       Kind (flavor)?

- From dry mix, ready-to-eat, frozen on stick, or recipe? If dry mix or recipe, was it regular or sugar-free? What type of milk was used in preparation?

- Any additions (i.e., bananas in banana pudding, whipped cream)?

B . Gelatin

- Regular or sugar-free?

- Clear or whipped?

- Prepared with cream cheese? Fruit? If fruit, what kind (e.g., canned peaches, fresh banana)?

- Any additions (i.e., whipped cream)?

14. EGGS

- How were they prepared (i.e., fried, boiled, scrambled)?

- Fat and salt in preparation?

- Whole egg? White only? Yolk only?

- Brand name of egg substitutes? If brand name unknown, was it liquid or powder?

- Accompaniments?

15. FAST FOODS

- Name of fast food chain?

- Food item(s) eaten?

- Additions to food item (i.e., catsup on fries, lettuce or tomato on hamburgers, extra mustard or catsup, etc.)?

- Deletions of "extras" on a food item (i.e., a hamburger without the special sauce.)

- For hamburger and fries, what size (e.g., regular or junior, or regular or large)?

- For soda, what kind (i.e., Coke, Pepsi)? Regular or diet? Small, medium, or

large? With or without ice?

16. **FATS**

   A. Butter

   - Type (i.e., regular, whipped or butter/margarine blend)?

   - Salted or unsalted?

   B. Margarine

   - Brand name, if known.

   - Type (i.e., whipped, diet, spread, or butter/margarine blend)?

   - Form (i.e., stick, tub, or liquid)?

   - Salted, unsalted or low sodium?

   - Type of oil (e.g., corn, safflower)?

   C. Oils

   - Brand name or type of oil.

   D. Shortening

   - Brand name or type of base (i.e., animal, vegetable, or a combination)?

   E. Animal Fat

   - Kind?

17. **FISH AND SEAFOOD**

   - Kind?

   - How was it prepared?

   - Fat and salt in preparation?

   - Form for some types (i.e., fresh, frozen, canned, smoked, dried)?

- Was anything added before, during or after cooking, e.g., marinade or coating? If marinated, was it a soy sauce or non-soy sauce mixture? If coated, was coating eaten?

- For canned fish, in what was it canned (i.e., oil, water, tomato sauce)? Was the fish drained and/or rinsed? Regular or low sodium?

18. **FRUITS**

- Kinds?

- Form (fresh, cooked, canned, frozen, dried, juice)?

- For some fruits, with or without skin?

- Sweetened or unsweetened?

- For canned fruit, what type of syrup (i.e., water pack, juice pack, light or heavy syrup)?

- For juice, was it real? If real, what form (i.e., fresh, frozen, or ready-to drink) and sweetened or unsweetened?

- For cooked fruits, was anything added before, during, or after preparation, e.g., fried apple rings?

19. **GAME**

- Kind (i.e., antelope, rabbit, squirrel)?

- Was fat trimmed or not trimmed?

- How was it prepared?

- Was anything added before, during, or after cooking?

- If venison/deer, what cut?

- If bird (i.e., pheasant, quail, dove), skin eaten or removed?

20. **GRAINS**

- Kind of grain (i.e., rice, barley, bulgur)?

- Type of preparation (i.e., instant, quick, regular)?

- Fat and salt used in preparation?

- Anything added (i.e., gravy, fat)?

21. GRAVY

- Kind of gravy (i.e., beef, chicken, mushroom, onion)?

- Prepared from recipe, canned or dehydrated? Prepared with canned soup, skimmed broth, meat juices, or bouillon? If bouillon, regular or low sodium?

- Plain, milk base, or water base? If milk, what percent fat?

22. ICE CREAM

- Brand name?

- Type (i.e., regular, ice milk, dietetic, imitation)?

- Form (i.e., regular, soft serve or a stick)?

- Flavor?

- Any additions (i.e., cone, topping, whipped cream, nuts)?

- For milkshakes, flavor and ingredients (e.g., hard ice cream, soft serve ice cream, nondairy fast food "thick shake")?

23. LAMB

- What cut?

- Was fat trimmed or not trimmed?

- How was it prepared?

- Was anything added before, during, or after cooking, e.g., marinade or coating? If marinated, was it a soy sauce or non-soy sauce mixture? If coated, was coating

eaten?

-    Fat and salt added in preparation?

24.   **LUNCHMEATS**

-    Brand name?

-    Kind (e.g., bologna, ham, salami, frankfurter)?

-    Meat base (e.g., chicken, beef, pork)?

-    Form (e.g., canned, loaf, thin sliced, spread, minced, chopped)?

-    Regular or low sodium?

25.   **MILK**

-    Kind (i.e., white, chocolate, buttermilk, substitute)?

-    Type (i.e., whole, 2%, 1%, skim)?

-    Form, (i.e., regular, evaporated, dry, condensed)? If evaporated, was it diluted or undiluted?

-    Flavorings or additions (i.e., egg, malt, sugar, chocolate)?

-    Brand name for milk-based breakfast or diet mixtures.

-    For milk substitutes, ask base (i.e., soy-based, filled saturated fat).

26.   **MIXED DISHES**

A .   **Mixed Dishes**

-    Name of mixture (e.g., chili, beef stew, macaroni and cheese).

-    Recipe or commercial (i.e., dry mix like tuna helper or frozen entrees)?

-    Main ingredient (e.g., beef, chicken, noodles, tuna). Obtain information on main ingredient in the manner outlined under appropriate food group.

-    Additional ingredients (i.e., vegetables, cheese, sauce, or gravy)? Obtain

information as directed above.

- Fat and salt in preparation?

- Any additions?

B. Pizza

- Thin, thick, French bread, or double (priazzo) crust?

- Toppings (e.g., pepperoni, cheese, sausage, olives, mushrooms)?

27. NOODLES

- Name (i.e., macaroni, spaghetti, noodles)?

- Type of pasta (i.e., white, wheat, spinach, egg)?

- Fat and salt in preparation?

- Any additions after cooking?

28. NUTS AND SEEDS

a. Nuts

- Kind

- Type (i.e., raw or blanched; dry, oil, honey roasted; sugar or chocolate coated, or a nut butter)?

- Salt or unsalted?

- Nut mixture (e.g., mixed with dried fruit)?

b. Seeds

- Kind?

- Salted or unsalted?

- Whole (unshelled) or kernels (shelled)?

- Type (i.e., raw, dry or oil roasted)?

29. **ORGAN MEATS**

- Name and from what animal (e.g., beef liver or pork brains)?

- Was fat trimmed or not trimmed?

- How was it prepared?

- Was anything added before, during, or after cooking?

- Fat and salt added in preparation?

30. **PIES**

A. Pies/tarts

- Kind (e.g., apple, lemon, chocolate)?

- Single or double crust?

- Regular or individual size?

- Prepared at home or commercially prepared?

- Type of fat used in crust?

- Type of fat used in filling (if appropriate)?

- Additions (i.e., meringue, whipped cream, ice cream, cheese)?

B. Turnovers

- Baked, fast food, or fried? If fast food, get name of establishment. If baked, get flavor. If fried, get type of fat used in frying? Prepared commercially or at home. If commercial, get brand name (e.g., Hostess).

Cobblers/crisps

- Flavor?

- Type of topping (i.e., streusel, pastry, biscuit)?

- For cobbler, type of fat used in topping? For crisps, type of fat used in recipe?

- Additions, (e.g., ice cream, cheese, whipped cream)?

31. PORK

- Was it a steak, roast, chop, or ground?

- What cut?

- Was fat trimmed or not trimmed?

- Fresh or cured?

- How was it prepared?

- Was anything added before, during, or after cooking, e.g., marinade or coating? If marinated, was it a soy sauce or non-soy sauce mixture? If coated, was coating eaten?

- Fat and salt added in preparation?

32. POULTRY

- What part of it light or dark meat?

- How was it prepared?

- Was anything added before, during or after cooking, e.g., marinade or coating? If marinated, was it a soy sauce or non-soy sauce mixture? If coated, was coating eaten?

- Fat and salt added in preparation?

- Cooked with skin or without? If with skin, skin eaten or not eaten?

33. SALAD DRESSING

- Brand name or kind if brand if unknown?

- Commercial or homemade? If homemade, type of fat used in preparation?

- Low calorie? Low sodium?

- Clear, creamy, or tomato base?

34.   SALADS

A.   Tossed

- Major ingredients (i.e., lettuce, spinach)?

- Raw, cooked, canned, or marinated? If cooked, from fresh or frozen? If canned, regular or low sodium?

- For cooked, marinated, and canned, get fat and/or salt used in preparation.

- Additional ingredients (i.e., dressing, cheese, croutons, bacon bits)?

B.   Fruit Salad

- Kind of fruit?

- Fresh, frozen, or canned?

- With dressing? What kind (mayo-type, whipped cream, etc.)

- Additional items (i.e., nuts, cream cheese)?

C.   Other Salads

- Type, (i.e., tuna, macaroni, egg, potato, coleslaw)?

- With or without eggs?

- Major ingredients (i.e., meat, cheese, etc.)? Obtain information on main ingredient in manner outlined under appropriate food group.

- Type of fat used in preparation?

35.   SAUCE

- Name (i.e., Bearnaise, cheese, hollandaise, steak, soy, spaghetti)?

-    Homemade or commercial, where applicable?

-    Ingredients, where applicable (i.e., meat or meatless spaghetti sauce, green or red enchilada sauce, or salsa)?

-    Type of fat used in recipe?

-    Regular ow low sodium, where applicable (i.e., soy or tomato sauce)?

36.   **SAUSAGE**

-    Brand name?

-    Pork, beef, or other?

-    Fresh, smoked, or brown and serve?

37.   **SNACKS**

-    Brand name for cheese puffs, corn chips, microwave-type popcorn, granola bars, potato chips, party-type mixes and tortilla chips. Otherwise, ask name of item (e.g., cornuts, pretzels, etc.)?

-    Salted or unsalted?

-    For popcorn, method of preparation and whether plain, cheese flavored, or coated? If popped in oil, get type of oil. If commercially popped, was it "buttered" (butter-flavor) or not "buttered"?

-    Any additions such as dip or salsa to chips or butter and salt to popcorn?

38.   **SOUP**

-    Kind (e.g., onion, mushroom, asparagus, chicken noodle)?

-    Form (i.e., canned, dry mix, homemade)? If canned, regular, chunky, or low sodium? If condensed, diluted or undiluted? If diluted, what liquid was used? If milk, specify percent fat.

-    For cream soups, type of fat in preparation?

-    Any additions (e.g., crackers, croutons, etc.)?

39. **SPECIAL FORMULATED PRODUCTS**

A. **Bar/Wafer**

   - Brand name?

   - Kind (breakfast bar, diet meal, high protein)?

B. **Drink**

   - Brand name?

   - Kind (i.e., fluid/electrolyte replacement solution, low calorie gelatin beverage, low or high calorie milk beverages, meal replacement drink)?

   - For low calorie milk beverages, was it canned or prepared from a powder? What flavor?

   - For meal replacement drink, was it gelatin, milk, or soy based? High protein? Predigested protein?

C. **Protein Supplement Tablet**

   - No further probing necessary.

D. **Dry Unprepared Powder**

   - Brand name?

   - Kind (e.g., instant breakfast, high calorie, meal replacement, nutrient supplement, protein supplement)?

   - For instant breakfast, was it regular or sugar free? What flavor?

   - For meal replacements, was it diet (and fortified)? If so, what flavor? If not was it a gelatin base, soy based with herbs, or was it high protein (milk based)?

   - For nutrient supplements, was it regular or low calorie?

   - For protein supplements, was it a beverage? If so, was it regular or sugar

free? If not, was it low calorie, low lactose, milk or soy based or sodium controlled?

- Reconstituted with what type of liquid? If milk, specify percent fat.

40. SWEETENERS

- Kind of sweetener?

- For jams, jellies, and preserves: regular, low sugar, or dietetic?

- Types of sugar (i.e., white, brown, powdered)? If brown, was it crystal or liquid?
- Type of syrup (i.e., pancake, pure or mixture)? If pancake syrup, was it regular, low calorie, diet, maple flavor, buttered blend, or fruit flavor? If pure mixture, specify base(s).

- Brand name of artificial sweetener? If unknown, specify whether saccharin or aspartame; liquid or dry?

41. VEAL

- Was it steak, roast, chop, or ground?

- What cut?

- Was fat trimmed or not trimmed?

- How was it prepared?

- Was anything added before, during, or after cooking, e.g., marinade or coating? If marinated, was it a soy sauce or non-soy sauce mixture? If coating, was coating eaten?

- Fat and salt added in preparation?

42. VEGETABLES

- Kind?

- Form (i.e., raw, cooked, canned, or dehydrated)? If raw, was the vegetable plain or marinated? If cooked, from fresh or frozen? If canned, regular or low sodium?

- Method of preparation?

- Fat and salt in preparation?

- Anything added before, during or after preparation (e.g., fat, cream sauce, sour cream)?

- For potatoes, eaten with or without peel?

- For juices, kind? Was the juice regular or low sodium (as in tomato juice)?

43. **YOGURT**

- Brand name?

- Type (i.e., plain, fruited, flavored, with fruits and nuts, or frozen? If frozen, get form (i.e., sandwich, on a stick, coated bar) or flavor?

- Made from whole, low fat or nonfat milk?

- Any additions (e.g., toppings, sweeteners, or fruit).

Participant's ID Number (SHS)	Date of Visit — mo day yr	Social Security Number	Date of Birth	Sex: 1=Female 2=Male

Participant's Name

_____

_____

Initials

Interviewer's ID

Intake day 1 = Sun 2 = Mon 3 = Tue 4 = Wed 5 = Thur 6 = Fri 7 = Sat	Interviewer's opinion of information 1 = Reliable 2 = Unable to recall one or more meals *3 = Unreliable for other reasons	Was amount eaten 1 = Typical *2 = Considerably less than usual *3 = Considerably more than usual	Did you take any supplements (*vitamins minerals, etc.*) ? 1 = no 2 = yes (*If "YES," describe under Food and Beverage.*)

Place of interview: 1 = Clinic, 2 = Home

Prepared: 1 = home 2 = restaurant 3 = other

Explain starred (*) items in the COMMENTS space below.

Line No	Time eaten a = am p = pm — Hour	Minute	Salt added in preparation 1 = no 2 = yes 9 = unknown — Food and Beverages	Amt.	Was fat added in preparation? 1 = no 2 = yes 9 = unknown (*Note type of fat used in description*) — Complete Description
01					
02					
03					
04					
05					

COMMENTS: (*Give line no. when appropriate*)

Line No	Prepared: 1 = home 2 = restaurant 3 = other / Time eaten a = am p = pm					Salt added in preparation 1 = no 2 = yes 9 = unknown / Food and Beverages	Amt.	Was fat added in preparation? 1 = no 2 = yes 9 = unknown (*Note type of fat used in description*) / Complete Description
		Hour		Minute				
06								
07								
08								
09								
10								
11								
12								
13								
14								
15								
16								

ID

COMMENTS: (*Give line no. when appropriate*)

ID [   ]

Line	Prepared: 1 = home 2 = restaurant 3 = other					Salt added in preparation 1 = no 2 = yes 9 = unknown		Was fat added in preparation? 1 = no 2 = yes 9 = unknown *(Note type of fat used in description)*	
No	Time eaten a = am p = pm					Food and Beverages	Amt.		Complete Description
	Hour		Minute						
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									

COMMENTS: (*Give line no. when appropriate*)

ID [   ]

Line	Prepared: 1 = home 2 = restaurant 3 = other						Salt added in preparation 1 = no 2 = yes 9 = unknown			Was fat added in preparation? 1 = no 2 = yes 9 = unknown *(Note type of fat used in description)*	
No	Time eaten a = am p = pm						Food and Beverages	Amt.		Complete Description	
	Hour		Minute								
28											
29											
30											
31											
32											
33											
34											
35											
36											
37											
38											

COMMENTS: *(Give line no. when appropriate)*

2. QUALITY OF LIFE INTERVIEW

The objective of this study is to evaluate the Strong Heart Study participant's day-to-day functioning and well-being. The significance of this study can be summarized as following:

1. That there are needs and interests of obtaining data in regard to the quality of life in a population with a great burden of chronic conditions[1,2,3,4,5], and there is virtually no information about the quality of life in the Indian population.

2. With the amount of data that Strong Heart Study has collected, such as information about heart disease, hypertension and its treatment, diabetes and its control, use of medications, echocardiogram, pulmonary function, gallbladder, dietary intake, and various lab measurements, the potential use of the quality of life data is tremendous.

3. Through data linkage, the information collected from this study can be linked to the IHS database, thus providing the opportunity to study the quality of medical care outcomes.

4. The Strong Heart Study may be the first large-scale epidemiologic study, not only in the American Indian population, but also in the general population, which includes about 40% of healthy subjects to assess the quality of life and provides invaluable baseline data for other or future studies to compare with[14].

The potential contribution of the study of the quality of life may become one of the most interesting and important findings to the Indian community, its health providers, and health policy makers.

The Medical Outcome Study (MOS) 36-ITEM SHORT-FORM HEALTH SURVEY (MOS SF-36, appendix 5) questionnaire will be used in this study to collect the data. Originally the questionnaire was developed by the RAND Corporation for the Medical Outcome Study (MOS), and later being condensed and standardized by Ware and Sherbourne (1992). The MOS SF-36 contains 36 questions which covers eight areas, physical functioning, role limitations due to physical problems, social functioning, bodily pain, general mental health, role limitation due to emotional problems, vitality, and general health perception. It was designed as self-administer questionnaire, and should not take more than five minutes to complete. If scoring slightly differently, the results can be compared with data collected by the RAND 36-ITEM HEALTH SURVEY 1.0.

The reasons the Rand 36-item Health Survey 1.0 was chosen as the tool to collect the data are based on the following reasons.

1. It is a generic (nondisease-specific), multi-item scale measuring each of eight health concepts: 1) physical functioning; 2) role limitations because of physical health problems;

3) bodily pains; 4) social functioning; 5) general mental health (psychological distress and psychological well-being); 6) role limitation because of emotional problems; 7) vitality (energy/fatigue); and 8) general health perceptions. Most of these items have been adapted from instruments that have been used for 20 to 40 years or longer[6], and all the items have been validated by various groups[3,7,8,9].

2.   The form is designed for self-administration, telephone administration, or administration by personal interview. It has been applied to general public[3]; participants who attended primary care facilities[7,9]; elderly low-income veterans[10]; patients with diabetes[11]; and patients who received hip replacement[12]. The age of participants ranged from 16 to over 80 years old. The response rates were about 85% in two of the studies administered by mailing the questionnaire to the participants[9,11], and over 95% of the respondents completed the questionnaire in one of the studies[9]. The average time to administer the questionnaire was 15 minutes for elderly veterans[10], while it took about 5 to 10 minutes for younger participants[6]. The questionnaire also has the precision to detect the difference of health status due to the different levels of severity of chronic medical conditions[7], due to the control of disease[11], or due to the treatment of medical conditions[12].

3.   The scoring system of the questionnaire is straightforward. All measures were scored on a scale of 0 to 100, with a higher score indicating a more favorable health status. First, assign this numeric score to each of the questions according to the answer chosen by the participant[13]. This score, between 0 and 100, represents the percentage of the total possible score achieved. Second, items in the same scale (i.e., each of the eight health concepts) are averaged together to create the scale scores; thus, eight scale scores will be created. Since the scores are treated as continuous variables, most of the parametric statistical methods can be used for data analysis. The eight scales can be analyzed individually or combined into different categories, depending on the purpose of the studies.

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THE STRONG HEART STUDY II

Quality of Life[1]

ID Number:

Social Security Number:

How is this questionnaire administered? (1=By interviewer, 2=By self, 3=Refused)

1. In general, would you say your health is: **(Circle One Number)**

    Excellent........................ 1
    Very good.................... 2
    Good 3
    Fair............................. 4
    Poor 5

2. **Compared to one year ago**, how would you rate your health in general **now**?
    (Circle One Number)

    Much better now than one year ago 1
    Somewhat better now than one year ago.............. 2
    About the same 3
    Somewhat worse now than one year ago 4
    Much worse now than one year ago 5

The following items are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

(Circle One Number on Each Line)

		Yes, Limited a Lot	Yes, Limited a Little	No, Not Limited at All
3.	**Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	1	2	3
4.	**Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1	2	3
5.	Lifting or carrying groceries	1	2	3
6.	Climbing **several** flights of stairs	1	2	3
7.	Climbing **one** flight of stairs	1	2	3
8.	Bending, kneeling, or stooping	1	2	3

---

1. Questions adopted from the RAND 36-Item Health Survey 1.0.

		(Circle One Number on Each Line)		
		Yes, Limited a Lot	Yes, Limited a Little	No, Not Limited at All
9.	Walking **more than a mile**	1	2	3
10.	Walking **several blocks**	1	2	3
11.	Walking **one block**	1	2	3
12.	Bathing or dressing yourself	1	2	3

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

		(Circle One Number on Each Line)	
		Yes	No
13.	Cut down the **amount of time** you spent on work or other activities...	1	2
14.	**Accomplished less** than you would like..................................	1	2
15.	Were limited in the kind of work or other activities.........................	1	2
16.	Had **difficulty** performing the work or other activities (for example, it took extra effort)...	1	2

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

		(Circle One Number on Each Line)	
		Yes	No
17.	Cut down the **amount of time** you spent on work or other activities	1	2
18.	**Accomplished less than** you would like.................................	1	2
19.	Didn't do work or other activities as **carefully** as usual....................	1	2

20. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

(Circle One Number)

Not at all...................... 1
Slightly 2
Moderately 3
Quite a bit.................... 4
Extremely 5

21.   How much **bodily** pain have you had during the **past 4 weeks**?

(Circle One Number)

None.............................	1
Very mild.....................	2
Mild	3
Moderate......................	4
Severe...........................	5
Very severe...................	6

22.   During the **past 4 weeks**, how much did pain interfere with your normal work (including both work outside the home and housework)?

(Circle One Number)

Not at all	1
A little bit	2
Moderately	3
Quite a bit	4
Extremely......................	5

These questions are about how you feel and how things have been with you during the **past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks** ....   (**Circle One Number on Each Line**)

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	None of the Time
23. Did you feel full of pep?	1	2	3	4	5	6
24. Have you been a very nervous person?	1	2	3	4	5	6
25. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
26. Have you felt calm and peaceful?	1	2	3	4	5	6
27. Did you have a lot of energy?................	1	2	3	4	5	6
28. Have you felt downhearted and blue?	1	2	3	4	5	6
29. Did you feel worn out?	1	2	3	4	5	6
30. Have you been a happy person?	1	2	3	4	5	6
31. Did you feel tired?...............................	1	2	3	4	5	6

32. During the **past 4 weeks**, how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

(Circle One Number)

All of the time...........	1
Most of the time	2
Some of the time........	3
A little of the time.......	4
None of the time........	5

How TRUE or FALSE is each of the following statements for you?

(Circle One Number on Each Line)

	Definitely True	Mostly True	Don't Know	Mostly False	Definitely False
33. I seem to get sick a little easier than other people.........	1	2	3	4	5
34. I am as healthy as anybody I know..........................	1	2	3	4	5
35. I expect my health to get worse............................	1	2	3	4	5
36. My health is excellent...	1	2	3	4	5

37. Interviewer's code

38. Date (mo/day/yr)

mo   day   yr

INSTRUCTIONS FOR USING RAND 36-ITEM HEALTH SURVEY 1.0

Interviewer should stress that "This part of the study is to determine **YOUR FEELINGS ABOUT YOUR HEALTH.** Your answers should be based on **YOUR** point of view. Try your best to answer ALL of the questions. If you really cannot decide what your answer is, just leave it blank and go to the next question. The importance of this study is that your feeling about your health can help us to find out how those feelings affect your other conditions, such as heart disease, high blood pressure, diabetes, and so on". After this opening introduction, ask the participant whether he/she want to answer the questionnaire himself/herself (self-administered) or have the questionnaire read to him/her (interviewer administered).

Tell participant if he/she has any trouble understanding any of the questions, don't hesitate to ask our staff for help. After completion of the questionnaire, thanks participant for participating in the Strong Heart Quality of Life Study.

Following are some examples of most often encountered problems in answering this questionnaire.

Q3 & Q4. **Vigorous activities**. Leisure: swimming, canoeing. Occupational: heavy construction, heavy farming - hoeing, digging, or mowing, chopping (ax), digging ditches, shoveling, sawing woods, hauling water, or any heavy industrial work.
Moderate activities. Walking, gardening, or ordinary household works. If participant asked about Indian dance, it should be decided by the pace of dancing. If it is fast paced (war dance or fancy dance), it would be vigorous. If is is slow paced, it would be moderate.

Q6 & Q7. **Climbing stairs**.
One flight is approximately walk up hill for 10 feet.
Several flights is approximately walk up hill for about 40-50 feet.

Q8. Difficult in **bending, kneeling, or stooping**.
The question meant 'either one of the condition', so that any of the three activities is limited by the current health status, the answer is "Yes, ...".

Q10 & Q11. **Walking Blocks**. One block is approximately 100 yards, **or** the length of one football field, **or** the length of three and half basketball court. Several blocks is approximately equal to half a mile (using British version of the SF-36).

Q23-Q31 How to choose an answer among "**Most of the Time, A Good Bit of the Time, Some of the Time, A Little of the Time**".
A little of the time: rarely, scarcely, seldom.
Some of the time: occasional, sometimes.
A good bit of the time: often, usually, frequently.
Most of the time: almost always.

3. STUDIES OF PSYCHOSOCIAL FACTORS

3.1 Rationale

There are vast amount of interest about the relationship between psychosocial and cultural attributes and the occurrence of cardiovascular disease. Since the Strong Heart population suffers a large burden of chronic conditions and facing rapid cultural changes, the Study provides a rare opportunity to explore these associations. In addition to life style, household income, education, and dietary intake, the Steering Committee decide to use standardized questionnaires to assess the level of acculturation, depression, interpersonal support, anger expression, and hostility in the Phase II examination. Due to the unfamiliarity and the difficulty to comprehend of these psychosocial questions in the Strong Heart Study communities, and, consequently, the unwillingness to answer the questionnaires, Arizona Center, which had the strongest opposition, decided not to participate this part of the study at all. Oklahoma and Dakotas would try on 200 participant to find out the community acceptance of these questionnaires. However, the Indian Health Services Institutional Review Board disapprove the use of these psychosocial questionnaires in the Strong Heart Study communities, this part of the study discontinued in Oklahoma and Dakotas promptly. Following is a sample of the introduction which will be read to the participant in explanation of why the Study needs these information and how the participant answer the questions.

3.2 A Sample of Introduction to the Participants

In the first phase of the Strong Heart Study, we assessed your health and health-related behavior that are known to be related to heart disease. It is also known that the interaction between our mind and body has an effect on our health.

In this current phase of the Strong Heart Study we want to study how stress and emotions may be related to heart disease. We have developed a questionnaire that we would like for you to complete to help us assess these factors.

This part of the study is voluntary just like other parts of the study. Your participation will help us to better understand the causes of heart disease in American Indians, and will shed light on the path to good health.

The questionnaire will show you your choices of answers for each question. Just write down the number for your answer. There are no right nor wrong answers, so please answer as best and as honestly as you can. Some of the questions may seem similar to you, but it is important that you answer each one as they have different meaning in our study. Please try to complete all questions, but if you have any question, please ask a Strong Heart Study staff member.

We want to remind you that all of this information is confidential. Your name will not be written on these forms, and personal answers will not be identified, only the group results will be considered.

You can read and complete the questionnaire by yourself, or a Strong Heart Study staff member can complete the questionnaire with you. Please let us know which way you prefer.

Thank you for your participation.

3.3   Cultural factors questionnaire

Reference: Oetting, E.R., and Beauvais, F. (1991). Orthogonal Cultural Identification Theory: The Cultural Identification of Minority Adolescents. The International Journal of the Addictions, 25 (5A & 6A), pp. 655-685.

Questions from: Denver Indian Social Health Survey. Denver Indian Health and Family Services, Denver, CO.

THE STRONG HEART STUDY II

CULTURAL FACTORS QUESTIONNAIRE

ID Number

Social Security Number

1. How is this questionnaire administered?
    1=By interviewer
    2=By self
    3=Refused

The next several questions are about your own native lifestyle.

2. How much do you identify yourself with your own native culture?
    1 =Not At All
    2 =A Little
    3 =Some
    4 =A Lot

3. How much do you identify yourself with non-Indian culture?
    1 =Not At All
    2 =A Little
    3 =Some
    4 =A Lot

4. How comfortable do you feel in your own native culture?
    1 =Not At All
    2 =A Little
    3 =Some
    4 =A Lot

5. How comfortable do you feel in the non-Indian culture?
    1 =Not At All
    2 =A Little
    3 =Some
    4 =A Lot

6. Interviewer's code

7. Date completed (mo/day/yr)

3.4   Center for Epidemiological Studies Depression scale (CES-D)

Reference Radloff, L. The CES-D Scale: A self-report depression scale for research in the general population. Applied Psychosocial Measurement, 1: 385-401, 1977.

Depression and Health A vast research literature exists establishing to varying extents the relationship between depression and health. Measurements of depression can be useful to assess the association of depressive symptoms with health risk behavior, prevalence and incidence of cardiovascular disease, and also to evaluate the effect of health status or CHD on mood states.

Assessment of Depression The CES-D was designed to measure current level of depressive symptomology, and especially depressive affect. The CES-D has been established as the "standard" for brief assessment of depression, i.e., in large scale epidemiological studies.

CES-D Utilized by Similar Studies The CES-D is the standard scale used in numerous large scale studies including the Honolulu Heart Program, the Inter-Tribal Heart Project (Menominee, Red Lake & White Earth), Cardia, and the Stanford Coronary Prevention Project.

Reliability and Validity The CES-D has been found to both adequate test-retest reliability, and internal consistency. The internal reliability (Cronbach's Alpha) of the CES-D is .89.

Administration Designed for self-administration, or interview format.

Scoring Twenty items are rated on a 4 point likert scale, ranging from "rarely, or not at all" scored as 1, to "most of the time" scored as 4. Four items are reversed when scored: #'s 5, 9, 13, and 17 so that 1 and 2 scores are changed to 4 and 3 respectively (and vice versa). Item scores are then summed for a total depression score (the higher the score, the greater the depression). Item #21 is not a part of the CES-D scale, and so should be scored separately.

Score Interpretation Upon completion of the survey, a staff member will sum the item scores, taking into account the reverse scored items. If the total score of items # 1-20 is above the CES-D cutoff score for indication of depression, the staff member is to ask the participant if they are interested in a referral for follow-up. The staff member then notes in the chart that the verbal offer of a referral had been given to the participant.

THE STRONG HEART STUDY II

CES-D SCALE

ID Number

1. How is this questionnaire administered?
    1=By interviewer
    2=By self
    3=Refused

Here are some questions (Q2-Q22) about your feelings during the <u>past week</u>. For each of the following statements, please respond as to whether you felt that way: Rarely or Not At All, Some of the time, Often, or Most of the time.

1	2	3	4	9
Rarely or Not At All (<1 day)	Some (1-2 days)	Often (3-4 days)	Most of the Time (5-7 days)	Not Applicable

During the **past week** . . .

2. I was bothered by things that don't usually bother me.

3. I did not feel like eating; my appetite was poor.

4. I felt that I could not shake the blues even with help from my family or friends.

5. I felt that I was just as good as other people.

6. I had trouble keeping my mind on what I was doing.

7. I felt depressed.

8. I felt that everything I did was an effort.

9. I felt hopeful about the future.

10. I thought my life had been a failure.

11. I felt fearful.

12. My sleep was restless.

13. I was happy.

14. I talked less than usual.

15. I felt lonely.

For each of the following statements, please respond as to whether you felt that way: Rarely or Not At All, Some of the time, Often, or Most of the time.

1	2	3	4	9
Rarely or Not At All (<1 day)	Some (1-2 days)	Often (3-4 days)	Most of the Time (5-7 days)	Not Applicable

16. People were unfriendly. □

17. I enjoyed life. □

18. I had crying spells. □

19. I felt sad. □

20. I felt that people disliked me. □

21. I felt like I couldn't do what I needed to do. □

For Question 22, please use the following scale

1	2	3	4	9
Rarely or Not At All	Some	Often	Most of the Time	Not Applicable

22. I have felt depressed or sad in this **past year**. □

23. Interviewer's code □□

24. Date completed (mo/day/yr) □□/□□/□□

3.5   The Interpersonal Support Evaluation List (ISEL)

References   Cohen, S., Mermelstein, R., Kamarck, T., & Hoberman, H. (1985). Measuring the functional components of social support. In I.G. Sarason & B.R. Sarason (Ed.s), Social Support: Theory, Research and Application. The Hague, Holland: Martinus Nijhoff.

Rosenberg, M. (1965). Society and the Adolescent Self Image. Princeton, NJ: Princeton University Press, pp.45-83.

Social Support & Health   Evidence of an association between stress and cardiovascular disease and many other psychological and physiological disorders has steadily accumulated over the years. Much research has focused on the role of social support in moderating the life stress-health relationship. In this context, the term "social support" refers to the various resources provided by one's interpersonal ties. The moderating effect of support on stress is often called the "buffering hypothesis". This hypothesis suggests that high levels of social support protect one from stress-induced pathology, but social support level is relatively unimportant for those experiencing low levels of stress. High social support has also been associated with lower levels of depression and other psychological disorders, lower physical symptomology, and with greater success in achieving and maintaining changes in health risk behavior such as smoking cessation and weight control.

Measure of Perceived Social Support This general form of the ISEL was designed to assess the perceived availability of four separate functions of social support as well as providing an overall support measure. Three of the four subscales will be utilized in this current study. The "Tangible" subscale is intended to measure perceived availability of material aid; the "Appraisal" subscale, measures the perceived availability of someone to talk to about one's problems; and the "Belonging" subscale measures the perceived availability of people one can do things with (socialize). This instrument has been widely used with adult populations of all ages (Health Psychology, 7:75-109, 1988). The "Self-Support" subscale was derived from Rosenman's Self-Esteem Scale, and measures self esteem and self support.

ISEL Utilized by Similar Studies The Inter-Tribal Heart Project (Menominee, Red Lake, White Earth) and the Honolulu Heart Program have utilized the ISEL in a number of different studies. Results suggest that social support is associated with prevalence and incidence of cardiovascular and pulmonary disease, as well as total and cause-specific mortality, disease prognosis, the utilization of medical care, and depressive symptoms. These findings are consistent with an expanding literature on the role of social support for stress reduction, health promotion/disease prevention.

Reliability & Validity The data demonstrates that the ISEL is a reliable measure of social support and that its subscales evidence reasonable independence from one another. The internal reliability (Alpha Coefficient) of the total ISEL scale is .88-.90. ISEL subscales range

from .73-.81 for Tangible Support, .70-.82 for Appraisal, and .73-.78 for Belonging,. The scale has good test-retest reliability, subscales ranging from .67-.84.

Administration This scale was designed for self-administration, or in interview format. Each item is to be answered on a 4 point likert scale where "Never True" is 0, "Rarely True" is 1, "Somewhat True" is 2, and "Definitely True" is 3.

Scoring The scale is scored so that a higher number (the more true) indicates more social support. Scoring must be <u>reversed</u> for several items so that a true response indicates support. Item reversals are: numbers 4, 9, and 14. When reversing items, 0 becomes 3, 1 becomes 2, 2 becomes 1, and 3 becomes 0.

THE STRONG HEART STUDY II

ISEL

ID Number

1. How is this questionnaire administered?
   1=By interviewer
   2=By self
   3=Refused

This scale is an assessment of social support, and is made up of a list of statements, which may or may not be true about you. For each statement (Q2-Q21), answer as to whether it is 'Never True', 'Rarely True', 'Somewhat True', or 'Definitely True' for you.

0	1	2	3
Never True	Rarely True	Somewhat True	Definitely True

2. If I needed a quick emergency loan of $30, there is someone I could get it from.

3. There is at least one person I know, whose advice I really trust.

4. If I needed help around the house (that is, with cleaning or making small repairs), I would have a hard time finding someone to help me without pay.

5. If I wanted to go play bingo, go to a potluck or pow wow, or some other activity, I could easily find someone to go with me.

6. I have a positive attitude about myself.

7. When I need suggestions for how to deal with a personal worry or problem I know there is someone I can talk to.

8. There are several people that I regularly enjoy spending leisure time with.

9. There is really no one I can talk to about money problems.

10. I have the confidence to do the things I want to do in my life.

11. If I needed help in doing some errands, I could find someone to help me.

12. I am a person of at least equal worth as other people.

13. I know someone that I can talk with about my most private thoughts and feelings.

14. If I needed a ride early in the morning, I would have a hard time finding anyone to take me.

For each statement , answer as to whether it is 'Never True', 'Rarely True', 'Somewhat True', or 'Definitely True' for you.

0	1	2	3
Never True	Rarely True	Somewhat True	Definitely True

15.   I often meet or talk with friends or members of my family. ☐

16.   I am basically a good person. ☐

17.   I often get invited to do things with others. ☐

18.   I feel satisfied with the help I get in doing tasks around the house, taking care of errands, and getting rides. ☐

19.   I feel satisfied with the amount of support I get with personal concerns. ☐

20.   I feel satisfied with how often I talk to, or get together with family and friends. ☐

21.   I feel satisfied with how I feel about myself. ☐

22.   Interviewer's code ☐☐☐

23.   Date completed (mo/day/yr) ☐☐/☐☐/☐☐

Reference   Spielberger, C.D., Johnson, E.H., Russell, S.F., Crane, R.J., Jacobs, G.A., & Worden, T.J. (1985). The experience and expression of anger: Construction and validation of an anger expression scale. In M.A. Chesney & R.H. Rosenman (Ed.s), Anger and Hostility in Cardiovascular and Behavioral Disorders (pp. 5-30). New York: McGraw-Hill and Hemisphere.

Anger & Incidence and Prevalence of CHD   Mounting evidence implicates the "AHA!" Syndrome (the triad of Anger, Hostility, and Aggression) in the etiology and pathogenesis of essential hypertension and coronary heart disease. Anger is most often defined as the emotional state that consists of feelings of irritation, annoyance, fury and rage, and heightened activation or arousal of the autonomic nervous system. Although hostility involves angry feelings, conceptually it is considered to be the cognitive component with the connotation of negative or cynical attitudes as well as chronic anger. Aggression generally refers to the behavioral expression of this anger and hostility directed toward other persons or objects in the environment. Since the concept of anger subsumes phenomena that are both more fundamental and simpler than the phenomena of hostility and aggression, anger is at the core of the AHA! Syndrome.

Previous research produced findings in which individual differences in the direction of anger expression were found to be associated with elevated blood pressure and hypertension (Harburg et. al. 1973, 1979; and Gentry et. al., 1981, 1982), and a common personality trait of individuals who develop CHD. Dembroski et. al., (1984) found that high ratings of potential for hostility and "Anger-In" were significantly and positively correlated with angiographically documented severity of coronary artherosclerosis. Other research has replicated similar findings (Williams et. al., 1980).

Measure of Expression and Experience of Anger   Originally, the Anger Expression Scale (AX) was designed to assess individual differences in anger expression as a personality trait, with the objective of investigating the role of anger in the etiology of heart disease. In assessing anger, the experience and expression of anger have to be separated. Anger feelings may be expressed in behavior, inhibited, or controlled in many ways. People differ in the extent to which they express their anger openly and in controling their anger.

The AX scale provides an assessment of overall "Anger Expression" (total score); an "Anger-In" score, which refers to how often angry feelings are experienced but not expressed (suppressed anger); and an "Anger-Out" score, which refers to the extent that an individual engages in aggressive behaviors when motivated by angry feelings.

Anger Expression Scale used by Similar Studies   The AX scale is considered to be one of the standard assessments of anger, and has been used in many studies, including

numerous prospective and cross-section studies of the roles of anger and hostility in CHD conducted by John Barefoot, et. al. at the Duke Medical Center. The Stanford Coronary Prevention Program has also used this scale extensively in both prospective and cross-sectional studies of the roles of anger and hostility in CHD.

Reliability & Validity Internal consistency (Alpha Coefficient) of the 20 item AX Scale and the 8 item "Anger-In" and "Anger-Out" subscales ranged from .73 to .84, and were highest for the Anger-in subscale. Overall, has established good validity and reliability.

Administration Can be self-administered, or in interview format.

Scoring The AX scales yields three scores: An Anger Expression score based on all 20 items, and scores for the 8-item Anger-In and Anger-Out subscales. Some of the AX items are worded in a manner such that a high rating indicates that anger is frequently expressed. Other items are worded so that a high rating indicates that anger is experienced but not expressed.

In calculating the AX total score, the scoring weights for items on which high ratings indicate the expression of anger correspond to the direct score. For items on which high ratings indicate that anger is not expressed, the scoring weights are reversed, i.e., the scores for responses marked 1, 2, 3, or 4, are changed to 4, 3, 2, & 1 respectively. To obtain the AX-Total score, simply sum the weighted scores for all 20 items, making sure to take the direction of scoring into account. In calculating the AX-Total score, the 9 directly scored items and the 11 reversed items are:

Directly scored items: 3, 5, 8, 10, 12, 14, 18, 20, 21
Reversed scored items: 2, 4, 6, 7, 9, 11, 13, 15, 16, 17, 19

The Anger-In and Anger-Out scores are obtained by summing the weighted scores for the 8 items that comprise each subscale. The scoring weight for each item corresponds to the number circled on the answer sheet; all 8 items are scored directly in calculating the subscale scores. The items which comprise the AX subscales are:

Anger-In: 4, 6, 7, 11, 13, 15, 16, 19
Anger-Out: 3, 8, 10, 12, 14, 18, 20, 21

The range of possible scores on the AX-Total can vary from a minimum of 20 to a maximum of 80. The range of possible scores on the Anger-In and Anger-Out subscales can vary from a minimum of 8 to maximum of 32.

THE STRONG HEART STUDY II

SPIELBERGER - AX

ID Number	

1. How is this questionnaire administered?
   1=By interviewer
   2=By self
   3=Refused

A number of statements which people have used to describe themselves when they feel __angry__ or __furious__ are given below (Q2-Q21). Please read each statement and then indicate how often you feel or act in the manner described when you are __angry__ .

1	2	3	4
Rarely or Never	Sometimes	Often or Always	Almost Always

When I feel angry . . .

2. I control my temper.

3. I express my anger.

4. I keep my feelings to myself.

5. I make threats I don't really mean to carry out.

6. I withdraw from people when I'm angry.

7. I give people "the silent treatment" when I'm angry.

8. I make hurtful remarks to others.

9. I keep my cool.

10. I do things like slam doors when I'm angry.

11. I boil inside, but I don't show it.

12. I argue with others.

13. I hold grudges that I don't tell anyone about.

14. I strike out (emotionally or physically) at whatever makes me angry.

Please read each statement and then indicate how often you feel or act in the manner described when you are <u>angry</u>.

1	2	3	4
Rarely or Never	Sometimes	Often or Always	Almost Always

15. I am more critical of (judge or find fault with) others than I let people know. ☐

16. I get angrier than I usually admit. ☐

17. I calm down faster than most other people. ☐

18. I say mean things. ☐

19. I am irritated (frustrated, annoyed) much more than people are aware of. ☐

20. I lose my temper. ☐

21. If someone bothers (frustrates, irritates) me, I am likely to tell him/her. ☐

22. Interviewer's code ☐☐☐

23. Date completed (mo/day/yr) ☐☐/☐☐/☐☐

3.7   Cook medley scale (HO)

References   Cynical Distrust - 9 Item Scale (8 items to be utilized) Greenglass, E.R., &
Julkunen, J., (1989)   Construct validity and sex differences in Cook-Medley Hostility.
Personality and Individual Differences, 10, pp. 209-218.

9-Item Scale also referenced in: Greenglass, E.R., & Julkunen, J., (1991). Cook-Medley
Hostility, Anger, and the Type A Behavior Pattern in Finland. Psychological Reports, 68, pp.
1059-1066.

Full Cook Medley Hostility Scale Reference: Cook, W., and Medley, D. (1954).
Proposed Hostility and Pharisaic virtue scales for the MMPI. Journal of Applied Psychology, v.
38., no. 6, pp. 414-418.

Hostility and Coronary Heart Disease Numerous prospective and cross-sectional
studies have found an association between hostility and a number of health outcomes including
Coronary Artery Disease, CHD events, Peripheral Artery Disease, cardio reactivity, hypertension,
risk factor status, and premature mortality from all causes. Not all results are conclusive, but
overall results   suggest the importance of assessing the relationship between hostility and
Cardiovascular Disease.

Assessment of Hostility The original Cook-Medley Hostility Scale (*Ho*) is a 50-item
subscale of the Minnesota Multiphasic Personality Inventory (MMPI), that measures cynical
hostility.

The Strong Heart Study will be utilizing the brief 9-Item Cook Medley Hostility scale
developed by Greenglass and Julkunen (1989). Specifically, this brief scale is a measure of
cynical distrust. One item has been deleted, thus 8 items will be utilized in this scale.

Some problems associated with self-report methods of hostility include the participants'
hesitation to admit to hostile patterns based on perception of social undesirability. Also, the *Ho*
assesses hostile "content", rather than "process" which can best be measured by direct observation
of verbal and non-verbal interactional styles. This latter form of assessment can be best achieved
through a method called the "Structured Interview", which although is a more effective and
accurate assessment of hostility, takes more time and requires more highly trained data collectors
and scorers, thus often rendering it unfeasible to use in large scale epidemiological studies.

Cook-Medley Utilized in Similar Studies Currently, the Ho scale is being utilized
extensively in studies including Cardia, Inter-Tribal Heart Project, Duke Medical Center research,
as well as numerous other research projects.

Research findings include: *Williams, et. al., (1980)*: Ho scores were associated with angiographically documented CAD in a clinical population.

Shekelle, et. al. (1983): In a prospective study of Western Electric Workers, a 10 year incidence of major CHD events related to *Ho* scores even after statistically adjusting for effects of traditional risk factors. In the 20 year follow-up, Ho scores predicted all cause mortality.

Siegman, et. al. (1987): Found relationship between *Ho* scores and angiographically documented CAD in patients under 60 years of age, but not in those over age 60.

Barefoot et. al., (1983): Ho scores significantly predicted incidence of CHD over 20 year period in 255 physicians. Ho scores were also related to mortality from a variety of causes including heart disease and cancer, and all cause mortality.

Barefoot et. al., (1989): In a prospective study, Ho scores were related to CHD and all cause mortality. Those at one standard deviation above the mean had an estimated 5.4. times the risk of dying than those at one standard deviation below the mean. The shorter 3 subscale version of Ho was found to be more predictive and to have a 50% greater chi square than the full scale.

Reliability and Validity The *Ho* scale appears to be satisfactorily valid and reliable. The authors report Internal consistency as estimated by analysis of variance, of .86, and test-retest correlation after 1 year was .85. The correlation between two ratings 4 years apart was .89.

Barefoot et al. (1983) obtained a 1 year test-retest correlation of $r = .85$, and Shekelle et al. (1983) reported a similar figure ($r = .84$) over 4 years. Thus, the *Ho* scale apparently assesses a very stable characteristic.

Administration Designed for self-administration, or interview format.

Scoring This 8 Item scale is to be answered True, False, or Don't Know (N/A if the participant is not willing to answer the question). The score on the scale is the total number of items marked true (indicating hostile direction).

THE STRONG HEART STUDY II

COOK MEDLEY

ID Number

1.   How is this questionnaire administered?
      1=By interviewer
      2=By self
      3=Refused

These next questions (Q2-Q9)are about how you think about other people. Although we cannot really know what other people would think or do unless they tell us, we would like to know your opinion as to whether you think each of the following statements is "True" or "False".

        0   1
      False   True

2.   No one cares much about what happens to me.

3.   It is safer to trust nobody.

4.   Most people would lie to get ahead.

5.   Most people inwardly dislike putting themselves out to help other people.

6.   Most people will use unfair means to gain an advantage rather than lose it.

7.   Most people are honest mainly through fear of being caught.

8.   I often wonder what hidden reason another person may have for doing something nice for me.

9.   Most people make friends because friends are likely to be useful to them.

10.   Interviewer's code

11.   Date completed (mo/day/yr)

3.8   Risk factor knowledge items

Reference National Center for Health Statistics (1985). *National Health Interview Survey* . Health Promotion / Disease Prevention section.

Previous studies have focused on the relationship between knowledge and perceptions of health, and health risk behavior. Results suggest that an individual's perception of related risk may influence whether or not they will adopt self-protective behaviors. These findings highlight the importance of assessing the relationship between individual and group knowledge of health-related risk factors and heart disease. Although knowledge of risk factors does not necessarily motivate an individual or group to modify their health-related behaviors, it does increase the likelihood. This evaluation can be useful in guiding individual and group health promotion and disease prevention programs for the modification of risk factors.

Item numbers 1-8 were developed for the *National Health Interview Survey* (1985). They are also being used in the Inter-Tribal Heart Project. In addition, item number 9 was added to assess knowledge of sedentary lifestyle as risk factor.

THE STRONG HEART STUDY II

RISK FACTOR KNOWLEDGE QUESTIONS

ID Number ⬜

1. How is this questionnaire administered? ⬜
   1=By interviewer
   2=By self
   3=Refused

This is a list of things which may or may not affect a person's chances of getting heart disease. After you read each one, answer as to how much you think it affects a person's chances of getting heart disease.

0	1	9
Does Not	Increases	Don't Know
Increase Risk	Risk	/Not Sure

2. Cigarette Smoking? ⬜

3. High Cholesterol? ⬜

4. High Blood Pressure? ⬜

5. Diabetes? ⬜

6. Worry, Anxiety, or Stress? ⬜

7. Being very overweight? ⬜

8. Eating a diet high in animal fat? ⬜
   (For example, foods that contain red meat, cheese, butter, lard, etc.)

9. Family history of heart disease? ⬜

10. Not exercising regularly? ⬜

11. Interviewer's code ⬜⬜⬜

12. Date completed (mo/day/yr) ⬜⬜/⬜⬜/⬜⬜