HEIRS
Manual of Procedures
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Hemochromatosis and Iron Overload Screening (HEIRS) Study
Manual of Procedures Introduction

Purpose

This Manual of Procedures provides the implementation details for the Hemochromatosis and Iron Overload Screening (HEIRS) Study Protocol. The Protocol, or study design, is provided as an appendix to this document. Two copies of this Manual of Procedures have been provided to each Field Center and one copy to the other HEIRS Study agencies for reference and daily use as a tool for quality control measures. Additional copies may be reproduced at each center as needed.

This document constitutes the Version 2, May 2002, Manual of Procedures (MOP). This manual has been updated from the previous May 2001 version. Changes to the study procedures themselves have been approved by the HEIRS Steering Committee. The updated pages/sections will be distributed along with instructions for removing old pages and inserting the new ones, as well as a log of the revisions. The MOP undergoes revisions in accordance with the needs of the study. Once the MOP has been finalized, it will be available to the scientific community upon request to the CoC for $US100 plus shipping charges.

Background

Iron overload is a common health problem mistakenly believed by many to be quite rare. The disease is insidious in onset, and many or even most individuals diagnosed with this disorder are not identified until advanced organ damage is present. Excess iron is deposited in body tissues, and can accumulate to toxic levels over time, leading to cirrhosis of the liver, hepatocellular carcinoma, congestive heart failure and arrhthymias, diabetes, arthritis and sexual dysfunction. Men tend to exhibit complications at an earlier age than women do since the latter maintain naturally lower iron levels through menstruation and pregnancy. While patients may manifest signs and symptoms of these complications, the underlying iron overload may not be recognized. Iron overload is relatively easy to treat by removing the excess iron through repeated phlebotomy. In addition, evidence suggests that early diagnosis and treatment can prevent disease manifestations and enable normal life expectancy. Thus, hemochromatosis may be suitable for detection and intervention through primary care screening strategies because: 1) it is relatively common; 2) it is asymptomatic in its early stages; 3) screening methods are reliable; 4) standard diagnostic methods are widely available in developed countries and are relatively inexpensive; 5) it is easily and safely treatable; and 6) if untreated, the subsequent burden of morbidity and mortality is substantial. However, there are a number of uncertainties about screening for iron overload and hereditary hemochromatosis, including the prevalence of iron overload in diverse populations, the penetrance of genetic variations, and the optimal care and treatment of asymptomatic and/or genetically at-risk individuals. Consequently, the

feasibility and benefits of such a program remain to be assessed. It is also unclear whether to base early screening/diagnostic efforts on serum iron assay indicators (phenotypic measurement), genotyping assays, or a combination of both. The Hemochromatosis and Iron Overload Screening (HEIRS) Study will examine these and related issues.

Iron overload can be the end result of a wide range of genetic, hepatic, and blood diseases. Hereditary forms of iron overload have been identified in Caucasian and African populations. The most common type of iron overload seen in people of northern European ancestry is *HFE*-linked hemochromatosis, named after the discovery of the *HFE* gene (chromosome 6) in 1996. As a result of this discovery, a DNA-based genetic blood test became available and provides an opportunity for early and rapid genetic identification of individuals at risk for development of hereditary hemochromatosis. Much remains to be learned about the penetrance and expression of these alleles, including their relevance to the full spectrum of clinical disease. While 80-90% of Caucasian hemochromatosis patients have *HFE* abnormalities, there are hetero- and homozygotes that do not manifest any evidence of disease, or manifest disease at different ages and with different outcomes, implying the existence of other genetic or environmental factors. Similarly, not all hemochromatosis patients have HFE abnormalities. Other genes yet to be discovered are also likely to be involved in the pathogenesis of iron overload and familial hemochromatosis, particularly in non-Caucasian populations. *HFE* genotyping at an early age is not a definitive indicator of later disease. Labeling a patient as having a genetic disease or being at genetic risk or susceptibility to a disease also may have discrimination ramifications in terms of denial of medical and life insurance and related employment issues. Thus, consensus panels to date have recommended that universal screening should not be initiated until more is understood about the relationship between genotype, phenotype and hemochromatosis-related disease outcomes. Further study of minority populations is also essential in order to plan screening /early diagnostic programs appropriate for all groups.

The HEIRS Study is designed to study the prevalence, penetrance, genetic and environmental determinants and modifiers, and potential clinical, personal, and societal impact of iron overload and hereditary hemochromatosis, in a multi-center, multi-ethnic, primary care-based sample of 100,000 adults over a 5-year period. This information will be used to determine the feasibility and potential individual and public health benefits and risks of primary care-based screening and intervention for iron overload and hereditary hemochromatosis. Estimating the burden of preventable illness from unrecognized hemochromatosis is one of the most important of these needs. Determination of specific and sensitive early signs and symptoms of iron overload and hemochromatosis will enable earlier diagnosis and initiation of care to prevent sequelae. Comparing the relative value and acceptability of diagnosis and screening by genotype vs. phenotype means is also important, and may be relevant to other adult-onset genetic disorders as well. In particular, differences by racial/ethnic group, age and other characteristics will need to be examined to be sure the resulting recommendations are appropriate for all patients.

The specific objectives of the HEIRS Study are to:

a) Determine the prevalence in a primary care population, by race/ethnicity, of: 1) iron overload, defined as confirmed elevation of transferrin saturation and ferritin, and hereditary hemochromatosis; 2) demonstrable clinical and pathological abnormalities related to iron overload and hereditary hemochromatosis; and 3) genetic variants related to iron overload and hereditary hemochromatosis including the recently identified C282Y and H63D mutations of the *HFE* gene.

b) Identify risk factors influencing the phenotypic expression of iron overload and hereditary hemochromatosis in regard to demonstrable clinical and pathological abnormalities, and examine interactions between risk factors to determine the relationship between genotype and phenotype. Risk factors could include genetic factors (co-modifying genes), or non-genetic factors such as diet, gender, age, alcohol intake or hepatitis B and C viruses.

c) Examine ethical, legal and social issues related to the possibility of implementation of primary care-based screening for iron overload and hereditary hemochromatosis, including identification of appropriate health care delivery models and potential personal, societal, or family-related impact of and barriers to primary care- or population-based screening.

d) Estimate the heritability of iron overload and hemochromatosis, and initiate linkage studies to identify main effect and modifier genetic variants associated with iron overload and hemochromatosis.

Study Organization

The HEIRS Study was initiated and funded by the Genetic Epidemiology Scientific Research Group, Epidemiology and Biometry Program, Division of Epidemiology and Clinical Applications, NHLBI, in conjunction with the Blood Diseases Program, Division of Blood Diseases and Resources, NHLBI, and the Ethical, Legal and Social Implications (ELSI) Research Program, Division of Extramural Research, NHGRI, through a contract mechanism. There are five Clinical Field Centers, a Central Laboratory and a Coordinating Center (CoC). An organizational chart of the HEIRS Study and a list of participating centers appear in Appendices A and B of the MOP.

The HEIRS study has brought together a large team of experts in diverse disciplines such as medicine including hematology and gastroenterology, clinical genetics, population genetics, laboratory medicine, epidemiology, statistics, ethics, law, anthropology, psychology, and genetic counseling, to develop the protocol over a series of meetings that have taken place since the inception of the study in February 2000. The depth of this project is reflected in the detail of this Manual of Procedures and is evidence of the collective efforts of all of the HEIRS study team.

The HEIRS Steering Committee, composed of the Principal Investigators from each participating center, the ELSI Subcommittee Chair, the Project Officer (NHLBI) and a Steering Committee Chair, has responsibility for developing the protocol of the study, assisting the participating centers in the conduct of the study, tracking study progress and resolving operational issues. Specific protocol and operational issues are dealt with by the relevant Subcommittee. A list of Committee and Subcommittees, as well as their members, is provided in Appendix B of the MOP.

In accordance with NHLBI policy, a Monitoring Board consisting of experts in hemochromatosis/clinical medicine, genetic epidemiology, genetic screening, ELSI research, clinical laboratory issues, and epidemiology has been put together. The Monitoring Board will review and approve the HEIRS protocol and provide oversight of the study.

Protocol/Study Design Summary

The protocol, or study design, for HEIRS is provided in an appendix to the MOP. Appendix C of the MOP includes the study flow. A brief summary of the design is outlined below.

The HEIRS Study data collection will take place over three years and involve 100,000 male and female patients aged 25 and older, recruited from primary care clinics associated with five field centers in: Birmingham, Alabama; Irvine, California; London, Ontario, Canada; Portland, Oregon; and Washington, D.C. These clinics serve an ethnically and socioeconomically diverse sample of the primary care patient community; the recruitment goal is for inclusion of approximately 50% minority patients. The chosen clinics represent a variety of medical care systems including a health maintenance organization, a clinical blood collection center, public and private primary care provider offices and walk-in primary care clinics. Thus, resulting recommendations on early diagnostic screening for iron overload and hemochromatosis should be applicable to a wide range of patients and a variety of health care settings.

During the initial stage of the study, planned to begin in February 2001 and last until November 2002, primary care patients will volunteer to have blood drawn and answer a brief questionnaire on their demographics, reasons for participation and attitudes towards genetic testing (ELSI assessment). The blood specimens will be assayed for transferrin saturation, serum ferritin and *HFE* C282Y and H63D genotype. If other relevant polymorphisms, particularly those associated with African iron overload or other minority-prevalent iron disorders, are identified during the course of this study, additional genetic assays may be considered. Phenotypic and genotypic test results will be provided to patients, and if they have so consented, directly with their primary care providers. While this study will focus on excess iron, it is recognized that these screening assays may also lead to detection of previously undiagnosed iron deficiencies and anemias.

Patients identified as having genotypic or phenotypic evidence of iron overload and/or risk of hemochromatosis will be invited back for further diagnostic testing during the comprehensive clinical examination (CCE) phase. It is estimated that 1000 such patients will be identified. In addition, patients without iron overload or hereditary hemochromatosis, but who may have other types of disorders, will be invited to the CCE so as to ascertain which signs, symptoms, biochemical and genetic assays and complications are more commonly associated with hemochromatosis, and thus contribute to the recommendations on early diagnostic screening and testing. The CCE will assess iron stores, distinguish between primary and secondary causes of iron overload and examine the associated hepatic, endocrinologic, hematologic and cardiovascular disease correlates and sequelae of hemochromatosis. Blood specimens will be obtained for a variety of diagnostic biochemical assays including liver function enzymes, insulin, glucose, inflammatory markers, relevant viruses and complete blood count (CBC). A detailed family and medical history will be obtained. Additional questionnaires will used to determine lifestyle characteristics such as smoking status, alcohol intake, and diet assessments (particularly iron supplements and inhibitors and promoters of iron absorption) which may explain the range of expression of disease and pattern of complications in those genetically susceptible to iron overload and hemochromatosis. An extended ELSI assessment of issues related to genetic screening and testing will be conducted via mail, or in some cases, via telephone interviews after the CCE. This same ELSI assessment will be mailed to a group of patients with *HFE* variants other than C282Y homozygotes. CCE results will be provided to the patients, and again, if they have so consented, directly with their primary care providers.

Because of the genetic etiology of hemochromatosis, family members may also be at risk of disease. Thus, as examinees with evidence of primary iron overload are identified, eligible family members will be invited to receive a CCE. Besides directly ascertaining the disease status of these individuals both phenotypically and genotypically and referring for treatment as appropriate, their DNA samples will be used to identify modifier genetic variants related to the expression of iron overload and hereditary hemochromatosis disorders via genome scan genotyping and linkage analyses. If any additional relevant gene variants are found, additional patients at risk may be identified and will receive genetic counseling as appropriate.

Ethical considerations mandate that patients found to have evidence of iron overload or deficiency be treated to prevent or attempt to reverse clinical disease. As such, throughout the study, clinically relevant results will be provided to the patients, along with genetic counseling as appropriate. Patients will be encouraged to discuss these results and follow-up treatment options with their primary care provider. Definitive diagnosis of hemochromatosis may necessitate liver biopsy and/or quantitative phlebotomy. The decision for these tests should be made on an individual basis, involving the patient and his/her primary care provider, and will not be a part of this study. However, with the patient's permission, the results of these procedures will be obtained annually for inclusion in study analyses. Similarly, a one-time follow-up ELSI

assessment will be mailed to the CCE hemochromatosis patients. While treatment will occur outside of the study, the field center organizations are committed to providing these tests and follow-up care in the advent that the patient does not have adequate medical insurance.

1. RECRUITMENT

1.1 Overview

Effective recruitment and retention strategies are key to the success of this study. Success will depend on HEIRS study staff, as well as physicians and clinic staff. Effective recruitment methods include: developing recruitment materials that are socially and culturally appropriate for the groups being recruited; pilot-testing recruitment materials (e.g., brochures, consent form, posters, video); doing advance educational work in communities (e.g., local media, community leaders and organizations, community-gathering places, in-clinic posters); working with clinic staff and physicians to provide information about iron overload and this study, to address logistical issues (e.g., patient flow), and to get "buy-in"; providing written, and, if possible video, information about iron overload and this study; using a "train-the-trainer" model, providing training for recruiters that includes study explanation (plus FAQs), importance of respect for clinicians/staff and participants, and cultural awareness, as well as technical aspects of recruiting (such as subjective assessment of ability to give informed consent).

The target population is primary-care patients who are 25 years of age or older. The Field Centers' approaches to this target patient population differ, and involve approaching:

(a) Patients presenting at primary-care clinics (Howard University in Washington D.C., University of Alabama at Birmingham, University of California at Irvine)

(b) Patients presenting at clinical laboratories for a blood draw (London Health Sciences Centre in London, Ontario)

(c) Primary-care patients from a health-plan membership (Kaiser Permanente Northwest in the Portland, Oregon, metropolitan area, and Kaiser Permanente Hawaii)

The only other inclusion criterion for participation in this research study is ability to give informed consent.

Other volunteers will not be excluded from the HEIRS Study. The initial screening questionnaire was designed to capture information that will help us understand whether each participant is from the target population. Each FC may have more specific requirements about which individuals are eligible for the HEIRS Study. For example:

- at Kaiser Permanente sites, only people who are Kaiser Permanente Health Plan members are eligible.

Your local Manual of Procedures or local project staff will include more detailed information specific for your FC.

1.2 Pre-Recruitment Activities

There are three groups of people who are important to the HEIRS Study: potential participants, study staff, and clinic staff and clinicians. We will need to pay close attention to all three groups throughout the study.

In some populations, recruitment is enhanced by providing information about the study to the community. The more people have heard about a research study, the more comfortable they may feel about volunteering. Educational efforts may be particularly important for a condition like hemochromatosis, which many people will not be familiar with. Such efforts may include newspaper articles or radio spots about the HEIRS Study, iron overload, and hemochromatosis, and posters or brochures for recruitment clinics and labs.

Recruitment staff need to be well acquainted with the "big picture" of the study so that they can respond appropriately to questions from potential participants, as well as the details of the recruitment and screening visit protocols. Such information can be obtained through readings, didactic sessions, discussions, and/or practice recruitment sessions with HEIRS Study investigators or senior staff. Field Centers may also consider training that focuses on effective communication skills for brief encounters (e.g., brief negotiations), and ways to stay motivated day-after-day in the recruitment situation.

Good relationships with clinic/lab clinicians and staff are critical for maintaining access to our target population. We are guests in their clinical facilities. For many facilities, the primary concern will be the impact of our presence on their clinic flow. It is important to discuss with clinicians and/or clinic/lab managers their concerns and their ideas about how HEIRS Study procedures can best be implemented, and to monitor our impact on the clinics and clinic staff throughout the study.

1.3 Initial Recruitment Activities

Initial recruitment strategies differ across Field Centers, and include approaching potential participants by mail, over the phone, and in-person:

- Howard University: approach patients in clinics.
- Kaiser Permanente: mail information to members with upcoming visits and recruit in clinics; and mail information to members with recent visits and recruit those who respond with interest.
- London Health Sciences Centre: approach patients in blood-drawing lab and in physician offices.
- University of Alabama at Birmingham: approach patients in clinics.
- University of California at Irvine: call patients in advance of scheduled visits and recruit in clinics.

Recruitment materials include introductory letters, scripts to guide information-providing and consent processes, brochures or posters about iron overload, hemochromatosis,

and/or the HEIRS Study, and, at some Field Centers, an informational video. Your local Manual of Procedures or local project staff will include more detailed information specific for your FC.

1.4 Guidelines for Recruitment Strategy Components

Our intent is to create an image to potential participants that gives them confidence in the study and in us as individuals—that we are conducting the study in a scientifically sound and ethical way. In addition to being knowledgeable about the study, study staff need to dress and behave appropriately for the culture(s) prevalent in their areas. We need to use good customer service and interpersonal skills to manage conflict, resolve problems, and negotiate outcomes that meet everyone's needs.

For example, appropriate behavior includes addressing each potential participant by her/his title and last name (e.g., Ms. Smith or Mr. Yim), answering questions fully and openly, acknowledging when we need help answering questions and getting that help as soon as possible, and behaving in a non-judgmental manner to questions, concerns, and responses by potential participants. We need to stay calm and respectful, even if we are not addressed in that manner. If there are concerns or issues that you can't resolve with a (potential) participant, contact your supervisor or HEIRS Study staff designated by your FC's PI (e.g., the HEIRS Study project manager).

Potential participants may also judge us by how we treat our colleagues and clinic staff. We need to work together effectively by developing a high level of respect and cooperation within the team.

Dress should be within the norms for the work-site culture(s). This usually means that men need to wear nice pants and shirts, and that women need to wear nice slacks, skirts, or dresses. Our dress and jewelry should not call attention to ourselves.

Personal phone calls and food consumption should be limited to break times.

Your local Manual of Procedures may include more detailed information specific for your FC.

1.5 Eligibility Determination

There are two study-wide eligibility criteria for the screening phase:

1. Age 25 years or older
2. Ability to give informed consent

Age can be easily assessed by asking the potential participant her/his age. Individuals who are not yet 25 years old, but will be within the screening phase of the study, can be encouraged to contact study staff after reaching their 25th birthdays.

Assessing **ability to give informed consent** can be challenging. The general guideline is that an individual needs to have the cognitive ability and awareness to: understand what she/he is being asked to do; weigh the risk and benefits of participating; and

understand that participation is voluntary and her/his decision about participating will not affect her/his health care. However, cognitive awareness can change from day-to-day, and even minute-to-minute, especially among older people.

We expect that most people encountered in our recruitment efforts will be able to give informed consent—the age range will be quite broad, and most of the population will be ambulatory and independent. Recruiters will have to use their judgment when they are not certain a potential participant can give informed consent. Clues that a person may not be able to give informed consent include: accompanied by an individual who speaks for her/him; behaves in an erratic or unusual manner (e.g., talks angrily to others (not a specific individual) or to unobservable people); or seems confused about where she/he is or why she/he is at the clinic or lab. When uncertain, it may help to get a "2nd opinion" from another recruiter or clinic staff or speak with a supervisor.

Informed consent procedures are described in Section 2.4 Informed Consent.

Other eligibility criteria that are specific to your Field Center should be evaluated according to your local guidelines.

1.5.1 Ineligibility Criteria

Participants under the age of 25 and previous HEIRS Study participants are EXCLUDED.

1.6 Recruitment Reports

Up to date recruitment reports can be found on the "Reports" section of the HEIRS web site, located under "Initial Screening". The "Initial Screening" reports are divided into three types: overall recruitment, demographics, and lab results. The purpose of these reports is to provide each site with a real-time status report of their recruitment effort, and to assist the CoC and the Steering Committee in monitoring of each site's progress. The CoC will also produce recruitment reports showing the recruitment for each site compared to the goals for that site. There are numerous reports and plots available on the website, overall and site specific, including reports by race/ethnicity, age, gender and minority recruitment. These reports can be modified, and new reports generated, as the investigators of the study identify the need. Members can request modified or new reports using the "Report Request Board" feature of the web site. This is located under the "Reports" section, then it is listed as "Report Request Board." Examples of recruitment reports follow.

Table 1: Recruitment Log Summary Report

HEIRS Recruitment Rates by Field Center

Field Center	Number of Consents	New Participants (log form)	New Part./ Consents (%)	New Non-Participants (log form)	New Undecided (log form)	Recruitment Rate (%)
Overall						
U Alabama-Birmingham						
U California-Irvine						
Howard University						
Kaiser Permanente						
London Health Ctr						

Click Here for Charts by Time Period
Click Here for Graphics of Recruitment Rates
Click Here for Graphics of Recruitment Numbers

Table 2: Overall Recruitment Report

HEIRS Recruitment for Initial Screen

	Initial Screen			Screen Complete		
Clinic	Last 7 days^	Last 28 days^	Overall	Number Complete	Goal	Percent of Goal
Overall						
U Alabama-Birmingham						
U California-Irvine						
Howard University						
Kaiser Permanente						
London Health Ctr						

Last X days is based on the date of Informed Consent

Table 3: Demographics Reports

HEIRS Initial Screen
Race by Gender - All Participants

		GENDER	
Race/Ethnicity	TOTAL	FEMALE	MALE
Overall			
American Indian or Alaskan Native - Only			
Asian - Only			
Native Hawaiian or Pacific Isle - Only			
Black or African American - Only			
White - Only			
Two or more Races			
Unknown			

HEIRS Initial Screen
Race by Gender - Hispanic/Spanish/Latino

		GENDER	
Race/Ethnicity	TOTAL	FEMALE	MALE
Overall			
American Indian or Alaskan Native - Only			
Asian - Only			
Native Hawaiian or Pacific Isle - Only			
Black or African American - Only			
White - Only			
Two or more Races			
Unknown			

HEIRS Initial Screen
Hispanic Origin by Gender

		GENDER	
	TOTAL	FEMALE	MALE
Overall			
Hispanic/Spanish/Latino			
Non-Hispanic/Spanish/Latino			
Unknown			

1.7 Recruitment Tracking by Field Center Staff

People who decide not to participate or to discontinue participation can provide valuable information for assessing potential recruitment and retention problems. For people who express interest in the study, as evidenced by starting through the informed consent process, or who begin participation, we will collect qualitative information about reasons for not (or no longer) participating. The recruiter will record this information on the Daily Recruitment Log or Participant Follow-up Contact Log (see Appendix D) The reasons for refusing the CCE will be recorded, and data entered onto the website using the CCE Tracking system. These refusals reasons will be coded the same as on the Participant Contact Form, to include 12 numerical codes (no human contact, target person not available, no longer interested in study, elder/child care issues, temporarily unable to continue, permanently unable to continue, person moved, deceased or other refusals). (Please see the Data Management Chapter 10 for specifics on the Tracking System). These reasons will be reviewed at each site to determine whether there are issues that we can address to make study participation more feasible or desirable.

The primary aim of collecting and summarizing participation status data is to monitor the recruitment process. Two forms will be used for this purpose: (1) the Daily Recruitment Log and (2) the Weekly Recruitment Summary.

The data from these summaries will be used to estimate weekly participation rates. For purposes of monitoring recruitment, the participation rate may be defined as New Participants / (New Participants + New Non-Participants + New Undecideds). If the previous status of New Participants is recorded on the Initial Screening Form (e.g., answer to the question: 'Is this the first time you have been asked to participate in this study?'), then this information might be used to 'correct' denominators of participation rate estimates over longer time periods.

1.7.1 Daily Recruitment Logs (Revised December 2001)

Each recruiter will fill out a log to record participation information on individuals that he/she approached that day (see Appendix D). If a recruiter works at more than one clinic on a given day, then separate logs will be filled out for different clinics. Data from the Daily Recruitment Log will not be entered into the computer.

Completing the Daily Recruitment Log

Item by Item

- Field Center numeric code, Clinic numeric code, date (month, day, year), and Recruiter's ID code will be recorded initially. *(CoC will be assigning the FC and Clinic numeric codes to each FC).*
- All eligible persons in the clinic will be classified as a (1) Participant, or (2) a Non-Participant or Undecided and recorded in the pertinent column on the Log (columns 1-5). *A person may only have one classification.*

- The first column on the log is the age check, where recruiters can check immediately if the approached person is 25 years of age or older. Persons under the age of 25 do not need to be recorded on the log.
    - ◊ **Participants (Columns 2 and 3 on the Log)**: After ascertaining whether the person had been previously asked to participate in the study, the correct classification as a ***New Participant*** (a person who agrees on this date to participate and **has given written informed consent**) or as a ***Previous Participant*** (at an earlier visit, the person had already agreed to participate and has already provided written informed consent)) is recorded on the Recruitment Log.
    - ◊ **Non-Participants and Undecideds (Columns 4-6 on the Log)**: For persons who have not previously participated, once the person indicates no interest or is undecided in participating in the study, the recruiter will further classify this patient as a ***New Non-Participant***, ***New Undecided***, or Previous ***Non-Participant or Undecided***. A New Non-Participant is a person who had not been approached previously to participate; likewise, New Undecided is a person who had not been approached previously to participate. A Previous Non-Participant or Undecided is a person who had been asked at an earlier appointment about participating, and at that time, that person indicated that he/she either did not want to participate or was undecided about participation.
- *Optional Information* (Column 7) can be recorded in the appropriate space on the Log. Information such as an appointment time, Name, Phone, and Reason for not participating may be recorded.
- Ineligibles do not need to be included on the Daily Recruitment Log. The reason for this is that recruitment counts should reflect levels and rates of recruitment among only eligible individuals.

At the end of each day, the Recruiter may want to tally the number of classified persons (columns 2-6) in order to more easily and accurately record the summary on the Weekly Recruitment Summary, which will be data, entered. Remember, the Study Goal for recruitment is 100,000 participants!

1.7.2 Weekly Recruitment Summary Form

A Weekly Recruitment Summary form will be filled out weekly for each clinic based on the previous week's Daily Recruitment Logs within the clinic (see Appendix D).

Completing the Weekly Recruitment Summary Form

Item by Item

Field Center numeric code, Clinic numeric code, date (month, day, year), Number of Logs (i.e., the number of Daily Recruitment Logs that are summarized in the weekly log), and Beginning Date and Ending Date of Log are recorded initially.

Participation Category: The total weekly number for the following categories will be recorded by staff - (1) New Participants, (2) Previous Participants, (3) New Non-Participants, (4) New Undecideds, and (5) Previous Non-Participants or Undecideds.

Total Number: The total number of participants for each category is to be completed on the form in the Total Number Column.

After completing the Weekly Recruitment Summary, the Summary is entered in the HEIRS Web-based system. The importance of this data entry must be emphasized, as the study will keep track of this data.

1.7.3 Participant Follow-up Contact Log

A follow-up log will be completed by the clinic staff to track attempted contacts to participants after the Initial Screening Visit (see Appendix D). Data from this log will be used to categorize the following outcomes:

- There was no human contact made by **phone** (no answer to a phone call contact, an answering machine answered the call, or the phone was out of service/no forwarding number),
- No **household contact** was made (the person was not at home, person was deceased, person moved, the person was unable to continue temporarily, or the person was unable to continue permanently).
- The participant is **leaving the study** (no longer interested, elder/child care, ride/parking problems, did not like the involvement with the study, or other refusal)

Specific actions to be undertaken by the clinic staff for each outcome are described on the log, for example, re-trying a phone call on a different time/week, reading various scripts, or recording additional information.

1.7.4 Script for Use with Participant Follow-up Contact Log

A script will be used with the Participant Follow-up Contact Log for some of the outcomes mentioned above (see Appendix D). There is a script available for leaving a message, a condolences script, a thanks script for expressing appreciation for contribution to the study, and a help script for specific problems (i.e. parking). Clinic staff will identify the appropriate script to use.

2. THE INITIAL SCREENING VISIT

2.1 Overview

During the Initial Screening Visit, the HEIRS study will screen 100,000 patients during a regular visit to a primary care clinic or blood collection facility. Participants will be eligible for the screening phase of the study if they are age 25 or older and able to provide informed consent. Patients will be approached and asked to give informed consent. If they appear to have difficulty reading, reading of an oral script will be offered. Participants not appearing to comprehend the oral script will not be eligible. As a part of this visit, each participant will complete a Contact Information Form and an Initial Screening Form and have blood drawn. Clinic staff will review these forms for completeness and conduct phlebotomy. (Note: at the Kaiser Permanente sites, London, and some UAB sites, phlebotomy will be performed by non-study staff.) Both forms will be data entered locally. Blood will be shipped to the Central Lab, where values for transferrin saturation (TS) and serum ferritin (SF) will be obtained, a screen of the HFE gene for C282Y and H63D mutations will be performed, and blood will be stored for future use (if designated by the participant). The Central Lab will transmit data on TS, SF and HFE mutations to the CoC, and the CoC will make them available to the Field Centers (FC). (Note: at the London site, TS will be performed locally with Central Laboratory quality control.)

2.2 Preparation

Make the following preparations prior to approaching patients:

1. Forms

   Collect several sets of the forms that will be completed. Have the pre-printed study ID labels available for the forms. Collect the set of pre-printed Central Lab ID labels. Forms will be printed in English, Chinese (Mandarin), Vietnamese and Spanish. For each participant you approach, gather all the forms required for a visit, including the informed consent, Contact Information form, Initial Screening form, and Lab Test Request form.

2. Supplies and Equipment

   Blood draws may be performed in a different area than where the other Initial Screening Visit procedures are performed. Prior to the blood draw, set up vacutainer and aliquoting tubes on the racks and attach the pre-printed labels to the tubes. Place all phlebotomy supplies on the blood drawing table. A list of supplies is available in Chapter 13 of the Screening Collection and Processing MOP.

2.3 Visit Guidelines

The HEIRS Initial Screening Visits will be scheduled over approximately a two-year period, beginning February 2001 and ending November 2002. The visit will include an information form, a questionnaire and a blood draw. We estimate that the complete visit will require about 45 minutes. Clinics may vary the visit sequence to some degree, if needed, but the requirements listed below may not be altered.

1. Informed Consent
2. Participant Contact Information
3. Initial Screening Form.
4. Blood Draw (Lab Test Request Form).

2.4 Informed Consent

The initial step after recruitment is to obtain informed consent. This is a critical component of this study and sufficient time must be provided in order for adequate informed consent to be secured. Obtaining informed consent involves two components; *assuring that people are fully informed* about the research project and *obtaining written documentation of their agreement to participate* in the research. Specific informed consent is obtained at three points in time in the HEIRS study: at the initial screen, at the case/control study stage, and at the family study stage. This section addresses only the initial screening consent.

Explaining the form:

Recruiters should be fully familiar with the contents of the consent form. An oral script outlining the major content is provided in Appendix E. Recruiters may want to use some parts of the oral consent form to organize or supplement their description of the basic elements included in the consent form. Most notably, it directs participants' attention to the most important parts of the form.

The consent form contains all of the required elements of consent and describes in relatively easy-to-understand language (9^{th} grade reading level) the purpose of HEIRS study, what is expected of participants and in turn, what participants can expect from this study, most especially the initial screening study. While general information about subsequent stages of the study is provided in the initial screening consent form, participants should be told that agreeing to participate in the initial screening study does not assume their participation in later stages of the study. Recruiters should give a copy of the consent form to potential research participants so that they can read and consider participation in HEIRS. After a few minutes, the recruiter should check back with the potential participant to see if they have any questions. If there is any indication that the individual is having difficulty reading or understanding the form, the recruiter should offer to go over it with the participant.

Problems reading the form.

Some people may have difficulty reading the consent form. Recruiters should be aware of the average level of education of people coming into their clinics. If recruiters have a sense that reading is a problem, they can offer assistance in reviewing the consent form. However, it is possible that some people may take offense at the suggestion that they are unable to read, so this must be done cautiously. It is also permissible for participants to have a friend/relative assist them in reading or discussing the consent form. It is also permissible for people not to read the consent form themselves, but to listen to your explanation (which in that event should follow the details of the oral consent form). However, in all instances, the recruiter should be convinced that the potential participants are competent and that they generally understand the basic elements contained in the consent form. In addition, the participant must sign the consent form. If the recruiter believes that the participant lacks the capacity to understand or competence to consent, the patient is not eligible for the study. Should this occur, recruitment of that individual should be discontinued in such a way that it will not offend the individual.

Non-English Speaking Participants:

In some clinics, potential participants will not be English-speaking. Whenever possible, recruiters should be fluent in the language of the people seen in the clinic site from which participants will be recruited into the HEIRS study. At a minimum, recruiters should be somewhat familiar with the typical languages spoken in the clinics from which they recruit. Consent forms will be translated into Spanish, Vietnamese, and Mandarin, but not other languages. If communication with potential participants is not possible, those individuals should not be recruited into the HEIRS study. If there is any indication that English is not the potential participant's first language, recruiters should ascertain whether another language is preferred. If so, use the appropriate version of the consent form, and indicate the preference for an alternative language on the Contact Information Form. All other forms in the study should follow this preference. It is permissible for non-English-speaking participants to rely on a friend or family member as translator in reading and understanding the consent form and other forms as long as the recruiter is comfortable with this situation.

Documentation of consent:

The research participant must personally sign the consent form (or, if the participant is physically impaired but mentally competent, a legally authorized representative may sign for them). A staff person must sign the witness line. Signatures do not need to be notarized, and it does not matter if pencil or pen is used, although pen is much preferred. A copy or duplicate of the consent form must be given to the participant or their representative. The copy does not have to be signed. The signed and witnessed original consent form **must** be placed in the participant's study file.
Note: Local procedures for informed consent at Kaiser sites may vary.

2.5 Administration of Participant Contact Information and Initial Screening Forms

At the Initial Screening, the following forms will be completed by the participant after the informed consent: the Participant Contact Information Form and the Initial Screening Form.

2.5.1 Form 1 - Participant Contact Information Form

All participants at the Initial Screening Visit will complete the Participant Contact Information Form after completing the Informed Consent.

This form captures information about the participant's name, mailing address, and phone numbers, along with information about the participant's preferred time that a Clinic Staff person may contact the participant. The name and contact information of a friend is obtained. The friend may be able to provide information to Clinic Staff or contact the participant regarding HEIRS if Clinic Staff are unable to contact the participant. There is a space to record the participant's medical record number.

Form 1 asks if HEIRS may contact the participant's physician in order to provide the physician with the participant's test results. Contact information regarding the physician's name, address, and phone number may be recorded on this form.

The first page of this form also has space to affix the lab labels.

INSTRUCTIONS:

Page 1. Staff will complete the header information at the top of the first page of the form. This includes the participant id, acrostic, date of visit and the completer code. The participant will complete the remainder of the form. Assure the participant that this information is confidential.

Laboratory Labels

For Clinic Staff Use Only:

- Clinic staff will affix the lab labels on the appropriate spaces on the Participant Contact Information Form.

Identifying information

- Last Name, First Name
- Nickname (if applicable)
- Middle Name (if applicable)
- Title, i.e. Mr., Mrs., Miss, Ms., Dr. (if applicable)

Locating information

- Mailing Address (street address or P.O. Box)
- City, State/Province, Zip Code/Postal Code
- Home Phone Number, Work Phone Number, Cell Phone/Other Phone Number, E-mail Address
- Best day/time to contact you (day, time, and a.m./p.m.)
- Best number to call (home, work, cell/other)

Friend or relative who may be helpful in locating the participant if Clinic Staff is unable to reach the participant:

- Name of friend or relative who can reach you
- Home/Work Phone

Physician Notification of Results

Note: The participant's response here does not supercede the informed consent or your local procedure.

- May HEIRS contact the participant's physician with the test results? Indicate yes or no.
- If yes, record the following information:
  - ◊ Physician Name
  - ◊ Physician Address

Other identifying information:

- Medical Record Number

End of form - See Section 2.7 for forms processing and data entry.

2.5.2 Form 2 - Initial Screening Form (U.S. and Canadian Versions)

All participants at the Initial Screening Visit will complete the Initial Screening Form after the Informed Consent and Contact Information forms have been completed. The Initial Screening Form captures basic demographic information, including racial categories for U.S. participants; the Canadian version of this form contains identical information except for racial categories that are applicable to Canadians. The Initial Screening Form also captures information regarding participants' viewpoints about genetic testing and some general health questions.

INSTRUCTIONS:

Page 1. Staff will complete the header information at the top of the first page of the form. This includes the participant ID, acrostic, date of visit and the

completer code. The participant will complete the remainder of the form. Once completed, staff should review the form to ensure its completeness.

Gender - Participant checks appropriate box for gender.

Birth date - Participant enters birthday - month, day, and year - as two digit numbers (for example, April 8, 2000 is 04/08/00).

Descent - Participant checks appropriate box (yes or no) to indicate if Spanish, Latino, or Hispanic descent.

Racial broad categories - Participant checks appropriate box or boxes to indicate the broad racial category. *Note: The term "Hispanic" is being used as a description of cultural ethic background, not of race. Ask that Hispanic participants who question this, answer as best they can.*

How found out about study - Participant checks the appropriate sources/reason(s) to indicate how participant heard about the study.

First time asked to participate - Participant checks the appropriate box (yes or no) if this is the first time the participant had been asked to participate.

Medical conditions - Participant checks the appropriate box (yes or no or not sure) if a physician has informed the participant that he/she has any of the specified conditions (7a-f). Check to see that each item has been answered.

Note: Participation in the initial screen does not imply that the participant has iron overload or hemochromatosis.

Blood relatives having IO or HH or treatment by regular blood draws - Participant checks the appropriate box (yes or no or not sure) if any blood relatives have IO or HH, or have been treated by having regular blood draws. Blood relative is defined as parents, grandparents, brothers, sisters, half-brothers, half-sisters, aunts, uncles, and children.

9. For women only: Pregnancy status/breast feeding - check the appropriate box (yes or no or not sure) if the participant has been pregnant with the past 3 months or if currently breastfeeding.

10. Status of general health - Participant rates his/her health status by checking the appropriate box (poor, fair, average, good or excellent).

11. How True or False are the following statements (11a - 11d) regarding participant's health: Participant checks the appropriate box for each statement (definitely true, mostly true, don't know, mostly false or definitely false). Check only one box per statement.

12. How you feel and how things have been in last 4 weeks (12a - 12e) - Participant checks the appropriate box for each question (all of the time, most of the time, a good bit of the time, some of the time, a little of the time, and none of the time). Check only one box per question.

Note for questions 13-17: For participants who ask, clarify that we are seeking their opinion and there is not a right or a wrong answer.

The answers to questions 13 - 16 are strongly agree, agree, disagree and strongly disagree.

13. Information about genetic risk should be shared with family. Check only one box

14. Feeling about genetic testing to investigate disease risk is good - Participant checks the appropriate box.

Genetic testing is good because - Participant checks the appropriate box for each statement. Check only one box per each statement.(15a-15d).

Possible reasons why genetic testing is not good - Participant checks the appropriate box for each statement. Check only one box per each statement.(16a-16d).

Possible reasons why participant thinks people get sick - Participant checks the appropriate box for each statement (very important, somewhat important, not important and not sure). Check only one box per each statement.(17a-17e).

.End of form - See Section 2.7 for forms processing and data entry.

2.6 Blood Draw

Staff will use the Lab Test Request Form to record participant identifying information for the specimens. This completed form will be photocopied. Clinic staff will retain one copy of the Lab Test Request form in the participant's folder and the original will be sent with the lab specimens to the Central Laboratory.

The HEIRS Central Laboratory at the Fairview-University Medical Center is responsible for developing the protocols for blood collection, processing, shipping, and storage; writing manuals for operations; implementing laboratory testing quality control measures; training and certifying FC staff on these protocols; and performing assays and reporting results as specified in the contract. The blood samples of HEIRS will be collected and processed using a modified version of the protocol used for the NHLBI ALLHAT Study over the past 6 years. This protocol allows for efficient specimen collection, handling, storage, and shipment.

Written protocols are provided for the Screening Visit blood collection in Chapter 13. This is a very straightforward collection provided by clinical laboratories on a daily basis. The Central Laboratory will train the Study Coordinator for each FC in the protocols for processing and shipping of samples, as well as a written protocol designed specifically for the Initial Screening visit. For the Comprehensive and Family Study Exam blood collections, the Central Laboratory will provide a written protocol and central training for FC trainers. All technicians will be trained, evaluated, and certified prior to participation

in this part of the study. Recertification will take place periodically throughout the study. Performance will be monitored by the Central Laboratory and the CoC. Additional training will be initiated as necessary.

2.6.2 Blood Collection for All Visits

Blood collection will be performed using standard venipuncture (antecubital fossa in the arm) by trained staff. A total of 20 - 56 mL (equivalent of one to three tablespoons) of whole blood will be collected into two to five different collection tubes. Blood samples from the screening visit will be used for measurement of iron and genotyping for known hemochromatosis mutations. Serum and buffy coat will be stored in a long-term repository for future analyses. Blood samples from the comprehensive and family studies visits will be assayed for measures of liver function, alcohol consumption, diabetic status, inflammation, and viral markers (if liver enzyme tests are greater than 1.0 times the upper reference limit). Serum and buffy coat will be stored in long-term repository for future analyses. DNA may be isolated from blood cells collected at both the screening and comprehensive/family study visits and stored for future genetic testing related to iron overload disorders. Cells (i.e. lymphocytes) from the comprehensive and family study visit may be cryopreserved and used to establish cell lines for future genetic testing related to iron overload disorders.

The average length of time required for phlebotomy is expected to be 5 minutes.

Blood Collection Instructions:

The Lab Test Request Form will be completed during the participant's visit. A number of checkpoints related to QC will be addressed on the Lab Test Request Form for each blood draw.

Prior to venipuncture, the participant should be seated in the phlebotomy chair. The phlebotomist should assure the participant that this is a simple procedure, that only about one to three tablespoons will be collected and that no problems are anticipated. The tourniquet is then applied and venipuncture is performed as per instructions in the Manual of Operations.

After venipuncture, the venipuncture site is bandaged and the participant should rest in the chair. Any deviations to the protocol or adverse reactions should be noted on the forms either in response to specific questions or in the section for comments. Deviations or adverse reactions serious enough to be reported to the IRB must also be reported to the NHLBI Project Office.

2.6.3 Form 3 - Lab Test Request Form

Clinic staff will complete the Lab Test Request Form. Generally, the clinic staff will check the box as to which lab tests are to be done based on the visit, but the lab staff will need to complete the time of collection and the hours since last eaten. The exact

procedure may vary by FC. Laboratory specimens (blood draws) will be collected from all participants at the Initial Screening Visit and CCE. Family members participating in the family study will have laboratory specimens (blood draws) collected. This form will be sent along with the lab specimens and the clinic will retain a copy. See Chapter 13 for specifics about the instructions for blood draws and the types of analyses to be performed on these specimens.

INSTRUCTIONS:

Identifying information. **Generally, the Clinic staff will complete this part, but it may vary by FC.**

Specimen Collection Information (completed by Clinic Staff)

1. Clinic staff will complete the participant identifying header information at the top of the form (i.e. HEIRS ID number, acrostic, date of visit, and completed by code).

2. Lab Specimen ID number - A space is provided to affix the appropriate Laboratory ID bar coded number label provided by the Central Laboratory. Record the staff code for "Completed by". At the same time, affix an identical replicate Laboratory ID bar coded number label to the Participant Contact Form.

3. Clinic staff will check the gender box.

4. Date Specimen Collected - Record the date (month, day, year) of lab specimen collection.

5. Time of Specimen Collection - The phlebotomist will record the time when the blood draw was initiated (hour, minute) and time of day (a.m. or p.m.).

6. Hours Since Last Food - Record the number of hours that have elapsed since the participant last consumed food.

Labels Affixation Section

7. Clinic Staff check the box for the appropriate battery depending on the visit. On the Lab Test Request Form, the types and volume of blood collection tubes to be used are indicated.

8. It will be necessary for the staff to make a photocopy of the completed Lab Test Request form. One copy should remain in the participant's chart and the original should be sent along with the lab specimen to the Central Laboratory.

End of form

2.6.4 Re-Draw Blood Collection Policy

There are various situations when a participant may need to have their blood sample redrawn, such as inadequate or collapsing veins, participant time constraints, inadequate or compromised samples, etc. The policy for re-draws is as follows:

1) If samples have **NOT** been sent to the lab, destroy the compromised/damaged samples and proceed with the re-draw under the same lab id. The lab id may be hand written in this situation, paying close attention to legible writing of the numbers.

2) For complete or partial redraw of samples that **HAVE** already been sent to the lab, assign a new lab id to the portion being re-drawn. Affix the new lab id labels to a new lab test request form. Complete the participant identifying header information at the top of the form (i.e. HEIRS ID number, acrostic, date of visit, and completed by code). If no HEIRS id labels are available, the HEIRS id should be hand-written in the space provided, paying close attention to legible writing. Also record the date specimen collected, time collected, and hours since last food. The new lab id should then be data entered on the Initial Screen Informed Consent Form (for initial screens) or Clinical Assessment Form (for comprehensive clinical exams) in the space provided for secondary lab ids. In the check boxes to the right of the secondary lab id, indicate whether this is a partial or a complete redraw. Note the secondary lab id data entry field is only visible/accessible by the clinic coordinator, or the designated “data entry plus” personnel.

   If the problem is with a QC sample, do **NOT** re-draw.

2.6.5 Permanently Missing Lab Values

All efforts should be made for participants to have a re-draw of their blood samples. However, there will be instances where this is impossible, and results in permanently missing lab values. For complete or partial **permanently missing** blood samples, record which tubes are missing on the Initial Informed Consent (for initial screens) or Clinical Assessment Form (for comprehensive clinical exams) data entry screens on the HEIRS web site. This may only be data entered by the clinic coordinator, or the designated “data-entry plus” personnel. Missing values will be inserted at the CoC, and a "Letter 5" will be recommended in the data download destined for the local database. Any contact with the participant concerning the partial results will be at the field center's discretion.

Instructions for Replicate Samples: See chapter 13 on Lab Procedures

2.7 Forms Processing and Data Entry

Clinic staff should ensure that forms are processed in a timely fashion, from data collection to data entry and final storage of forms. Please refer to Chapter 10 for expanded data entry instructions.

1. Once the participant has signed the Informed Consent form and completed the Contact Information and Initial Screening forms, clinic staff should review them for completeness. **Be certain that the birthdate, gender and race/ethnicity questions have been answered.** Be sure to ascertain that the participant is at least 25 years old. Additionally, ask if they have previously participated to avoid duplicate participation. Participants who have previously participated are excluded. Fill in the header information for these forms and for the Lab Test Request Form. Using one row of three identical labels from the preprinted initial screening study id barcode sheets, attach a barcoded label to the Contact Information Form, the Initial Screening Form, and the Lab Test Request Form. Attach Lab id labels to the Contact Information Form and the Lab Test Request form: this ensures that the participant's questionnaire and laboratory data can be linked. (Note: in London Health Sciences Centre clinics, a blood sample is being sent to MDS, a local lab, and there is space on the Contact Information form for an MDS label as well as a Central lab label.)

2. If the participant is contributing a quality control duplicate sample, use a separate Lab Test Request Form, but attach the associated Lab id label to the Contact Information form belonging to the Participant donating the extra sample. Attach a study id label from the preprinted sheet to the Lab Test Request form for the quality control duplicate sample and **destroy the remaining two identical study ID labels remaining in the row.** This allows the lab to analyze the sample without knowing that it is a duplicate, but links the quality control results with participant they came from.

3. Upon completion of the Initial Screening Visit, staff should collect all the forms for each particpant, including the Lab Test Request form; these forms should be placed in a folder (labeled with the participant's identifying information, such as name, acrostic, or identification number) to ensure that forms will not be lost or misplaced prior to data entry.

4. If possible, clinic staff should review the forms while the participant is in the clinic rather than by a subsequent telephone call to retrieve or clarify responses or items that were left blank. A clarification for a blank or inappropriate response is easier to correct or obtain while the participant is in the clinic rather than a subsequent follow-up telephone call. Some questions may be sensitive and intimidating to a participant; therefore, the participant will intentionally leave some questions

unanswered. Use discretion in trying to obtain further information from the participant. It is important that we respect the participant's right to refuse to answer any questions that he/she chooses.

At the Initial Screening Visit, there are three mandatory items to be completed on the Initial Screening Form: birthdate, gender, and race. If these items were not completed by the participant, staff should retrieve this information prior data entry.

5. After reviewing forms and placing them in the participant's folder forward the folder to the data entry staff for entry in the computer. After entry, the folder containing the forms should be filed appropriately according to the clinic's discretion (alphabetical, numerically, etc.) for easy retrieval and filing of additional forms at a later date.

6. Data entry begins with the Contact Information form. Local procedures for use of this form vary but **it is required that the participant's name be entered using the local Contact Information form data entry application**. During data entry, an acrostic, or alphabetical code, will be generated for each participant. This acrostic provides an essential validation check for the study id that will be used if the participant returns for the Clinical Exam. The acrostic must be written on Contact Information and the Initial Screening form when it is generated. **Please be sure to copy the acrostic accurately!** When the Initial Screening form is data entered through the web site, the acrostic will have to be typed after the id label has been scanned. **Again, please be sure to do this accurately!** The acrostic will be confirmed when the information on the informed consent and preferred language is entered: after that, labels can be generated which include the acrostic, so that it will never have to be typed again, but the acrostic on the label must match what has been entered on the web site.

Chapter 3: PROCESSING RESULTS OF THE INITIAL SCREEN

3.1 Overview

Results from the Initial Screening Visit will include laboratory values for Transferrin Saturation (TS) and Serum Ferritin (SF), and tests for the C282Y and H63D HFE mutations. Based on these results, there will be two groups of participants that will be invited to attend the Comprehensive Clinical Exam (CCE) visit. The first group, the potential cases, will be invited because their genotype and/or phenotype results indicate they may already have or be at significant risk to develop iron overload in the future. The second group, the controls, will have phenotype and genotype test results within the usual range and will have been randomly selected to participate in the CCE. A third group of participants, the "ELSI-only" (sometimes referred to as Non-CCE ELSI) cases, will have at least one genotype or phenotype result outside the usual range, but not results that would qualify them for the CCE. They will be selected to be part of the ELSI assessment without participating in the CCE. The CoC began identifying these participants in February 2002, and the FCs are notified every Monday as to whom these participants are for the mailings. (Please see Chapter 7- for the ELSI substudy specific details).

3.2 Eligibility for Cases and Controls

Two possibly overlapping groups will be invited to the CCE: (1) all C282Y homozygotes and (2) other participants whose TS and SF values exceed both cutoffs in Table 1 below (no matter what their genotype result is). H63D homozygotes, heterozygotes, and compound heterozygotes who do not have TS and SF above the cutoffs will not be invited to participate in the CCE.

One important ongoing issue in this protocol is the CCE recall rate; that is, how many people are eligible and invited to participate in the CCE. Cutoffs may need to be adjusted as the study progresses, so that the recall number is controlled. Separate "alert value" cutoffs indicating a need for clinical follow-up will also be specified.

Table 1. TS and SF Cutoffs for Potential Cases

Lab Test	Males	Females
Transferrin Saturation	50	45
Serum Ferritin	300	200

Those participants in the Initial Screening Visit who are not eligible for the CCE as potential cases may be eligible as potential controls if their TS and SF values fall in the ranges specified in Table 2 and they have no C282Y or H63D mutations. The TS and SF ranges in Table 2 represent the first and third quartiles across all racial /ethnic groups in National Health and Nutrition Examination Survey (NHANES). Potential

controls will be selected to reflect the Field Center, gender, racial/ethnic and age distribution of the potential cases.

Table 2. TS and SF Ranges for Potential Controls

Lab Test	Males	Females
Transferrin Saturation	20 - 34	16 - 28
Serum Ferritin	87 - 247	29 - 121

For each participant, results of the TS, SF and HFE tests will be sent from the Central Lab to the CoC. The CoC will make them available to the Field Centers. Designated Field Center clinic staff should then inform each participant of the results of his/her TS, SF and HFE tests. Those individuals qualifying for the CCE as potential cases will be called, their willingness to participate further will be ascertained, and then an appropriate Results Letter will be sent. The telephone call to those individuals who may be cases and will be invited to the CCE should be used not only as an opportunity to discuss test results, but also secondarily as a recruiting tool. All other participants can be mailed their Results Letter as soon as results are available. Section 3.4 provides descriptions of the Results Letters to be sent to the participants.

3.3 Alert values

Some participants will have values for TS and SF that are below or above alert levels that have been specified for HEIRS. Table 3A contains the alert values that will be used initially, which are the 2.5th and 97.5th percentiles of TS and SF for the US population based on the NHANES III data. Two field centers (UCI and Howard) use alternative alert values because of regional differences in medical care (See Table 3B). Participants with alert values may have a clinical condition and should be evaluated by a physician, and they will receive a recommendation to contact their primary care physician as part of their results report. The ranges for alert values may change as the study progresses.

Table 3A: TS and SF Alert Values

Lab Test	Gender	Low	High
Transferrin Saturation	M F	15 15	50 50
Serum Ferritin	M F	25 15	530 300

Table 3B: TS and SF Alert Values at UCI and Howard only

Lab Test	Gender	Low	High
Transferrin Saturation	M F	15 15	50 50
Serum Ferritin	M F	25 15	440 205

The CoC, when providing these results, will include an indication of which participants have a TS or SF result in the alter range. The procedures for individual Field Centers vary regarding whether telephone calls will be made for participants with alert values. Results letters are described in the next section.

3.4 Description of Results Letters

The results of every participant's transferrin saturation, serum ferritin screen and HFE testing will be transmitted from the Central Laboratory to the Coordinating Center. Based on these results, a list of participant IDs with their results will be made available to the Field Centers through the HEIRS web site. An application on the HEIRS study PCs at each Field Center will indicate which Results Letter each participant should receive. The application will merge results data with the Participant Contact Information database on the HEIRS Study PC and the appropriate letters will be printed. It will be critical to confirm that the correct Results Letter is being sent to participants as this information may have significant implications for that individual's future health and well being. In addition, this will likely be the only written communication that most study participants will receive about their test results.

There are eleven different Results Letters. The letter a participant receives depends on three pieces of information: the participant's genotype, whether or not the participant has iron elevations by study definition, and whether or not the participant has an alert value. For those who are eligible for the CCE as potential cases, which Results Letter they are sent also depends on whether or not they agree to participate further. The following tables indicate which letter is sent for each set of conditions.

Tables 4a-d. Results Letters needed for each genotype, by iron elevations and alert status.

4a. No C282Y or H63D mutation detected

	Iron elevations	No iron elevations
No alert	1 or 1C	2
Low alert only	----	2A
High alert only	1 or 1C	2A
Both high and low alerts	----	2A

4b. C282Y homozygotes

	Iron elevations	No iron elevations
No alert	1 or 1A	1 or 1B
Low alert only	----	1 or 1D
High alert only	1 or 1A	1 or 1D
Both high and low alerts	----	1 or 1D

4c. H63D heterozygote

	Iron elevations	No iron elevations
No alert	1 or 1A	4
Low alert only	----	4A
High alert only	1 or 1A	4A
Both high and low alerts	----	4A

4d. Other HFE mutation combinations

	Iron elevations	No iron elevations
No alert	1 or 1A	3
Low alert only	----	3A
High alert only	1 or 1A	3A
Both high and low alerts	----	3A

3.4.1 Letters 1, 1A, 1B, 1C, and 1D

These letters will be sent to participants who are eligible for the CCE; that is, are C282Y homozygotes, have evidence of iron elevations (based on transferrin saturation), or both. All these participants will be contacted by telephone and invited to participate in the CCE. If they agree, they will receive Letter 1. If not, they will receive either Letter 1A, 1B, 1C or 1D as follows:

Letter 1A: This letter will be sent to those individuals who have:

1) NOT agreed to participate in the CCE;
2) have evidence of iron elevations (based on elevated transferrin saturation and serum ferritin); and
3) have either one or more of C282Y or H63D mutations.

Letter 1B: This letter will be sent to those individuals who have:

1) NOT agreed to participate in the CCE;
2) have **no** evidence of iron elevations (based on elevated transferrin saturation and serum ferritin) and no alert value; but
3) are C282Y homozygotes.

Letter 1C: This letter will be sent to those individuals who have:

1) NOT agreed to participate in the CCE;
2) have evidence of iron elevations (based on elevated transferrin saturation and serum ferritin); and
3) have no mutation identified.

Letter 1D: This letter will be sent to those individuals who have:

1) NOT agreed to participate in the CCE;
2) have **no** evidence of iron elevations (based on elevated transferrin saturation and serum ferritin) **but have at least one alert value;** and
3) are C282Y homozygotes.

3.4.2 Letters 2 and 2A

Letter 2: This letter will be sent to those individuals who:

1) are not eligible for the CCE;
2) have no evidence of iron elevations and have no alert values; and
3) have no mutation identified

Letter 2A: This letter will be sent to those individuals who:

1) are not eligible for the CCE;
2) have no clear cut evidence of iron elevations, but who do have alert values that require further follow up with their health care provider; and
3) have no mutation identified

3.4.3 Letters 3 and 3A

Letter 3: This letter will be sent to those individuals who:

1) are not eligible for the CCE;
2) have no evidence of iron elevations and have no alert values; and
3) have the following genotypes (C282Y heterozygosity; H63D homozygosity or compound heterozygosity)

Letter Three A: This letter will be sent to those individuals who:

1) are not eligible for the CCE;
2) have no clear cut evidence of iron elevations, but who do have alert values that require further follow up with their health care provider; and
3) have the following genotypes (C282Y heterozygosity; H63D homozygosity or compound heterozygosity

3.4.4. Letters 4 and 4A

Letter 4: This letter will be sent to those individuals who:

1) are not eligible for the CCE;
2) have no evidence of iron elevations and have no alert values;and
3) are H63D heterozygotes.

Letter 4A: This letter will be sent to those individuals who:

1) are not eligible for the CCE;
2) have no clear cut evidence of iron elevations, but who do have alert values that require further follow up with their health care provider; and
3) are H63D heterozygotes.

3.4.5 Letter 5 Incomplete or Missing Lab Results

This letter will be sent to those individuals with incomplete or missing lab results.

Letter 5: Occasionally, blood samples are compromised and may need to be redrawn. Some participants will have incomplete iron test results. An additional letter (Letter 5) will be sent in this case.

3.5 Procedures for Distribution of Results Letters and Education Materials

All participants in the initial screening exam will be notified of the results of their TS, SF and HFE screen. A copy of the actual laboratory results will be included in the letter sent to the participant. The study coordinator should double check that the information in the letter printed to go to the participant is consistent with the laboratory results to be included. If there is any question about whether the correct letter has been generated, the study coordinator should contact the PI of the Field Center or the study's genetic counselor to resolve their questions or concerns. Some site-specific variations in reporting procedures are identified in Appendix H. Most all participants fall within the usual reference range of the central laboratory and will be sent results by letter. Individuals in whom no genotype variations are identified and whose iron studies include no alert values will be invited to continue study participation as controls. Along with some of the results letters, educational materials about iron overload and hemochromatosis will be mailed to the participants. This includes Letters 1, 1A, 1B, 1C, 1D, 3, AND 3A. Letters 2, 2A, 4, and 4A will not be given the educational material on iron overload.

Upon receipt of complete lab results and data entry of the Initial Screening form, the CoC will generate a list of participants for each Field Center indicating which letter should be sent to each participant, and the participant's status for invitation to the CCE. The FC should fill out the Initial Screening Checklist (See Section 3.6) in preparation for notifying the participants. Field Center staff will print the appropriate letters and mailing labels for each participant through their local data base.

For those eligible for the CCE, an indication of whether the participant agreed to be scheduled for a visit will be used to identify which letter to print. In the letter or call, participants will be encouraged to discuss their results with their primary care provider particularly if they are not coming into the CCE and in the event that further evaluation for potential health risks and possible treatment is needed.

Field Center staff will obtain the appropriate signatures (Field Center PI or Clinic Coordinator and phone number) for each letter. Participants should be instructed to contact the Field Center (Clinic Coordinator/PI) in case clarification is needed for the letter.

3.6 HEIRS Initial Screening Checklist

HEIRS INITIAL SCREENING CHECKLIST

Participant ID _____ Acrostic _____

Date of Initial Screening Visit __ __/__ __/__ __

1. Initial Screening Informed Consent
   a. Consent signed: □ Yes □ No
   b. Date of signature: __ __/ __ __/ __ __

2. Changes in Initial Screening Informed Consent Status
   a. Date of change: __ __/__ __/ __ __ Withdraw from study? □ Yes □ No
   b. Date of change: __ __ /__ __/ __ __ Withdraw from study? □ Yes □ No

3. Preferred Language (check one)
   a. English ___ c. Mandarin ___
   b. Spanish ___ d. Vietnamese ___

4. Results Letter
   a. Results letter sent to participant: □ Yes □ No
   b. Which Results Letter was sent?

      _____

   c. Date results letter sent: __ __/ __ __/ __ __

5. Post Result Form
   a. Post Results Form mailed to participant: □ Yes □ No
   b. Date mailed: __ __/ __ __/ __ __
   c. Re-mailed: □ Yes □ No
   d. Date re-mailed: __ __/ __ __/ __ __
   e. Post Results Form received: □ Yes □ No
   f. Date received: __ __/ __ __/ __ __

6. Scheduling CCE

a. CCE scheduled: □ Yes □ No

b. Scheduled date of CCE: __ __ /__ __/ __ __

c. Participant refusal: □ Yes □ No

d. Reason for refusal: _____

e. Date of refusal: __ __/ __ __/ __ __

HEIRS CLINIC APPOINTMENT REMINDER and INSTRUCTIONS

ID # _____

Thank you for agreeing to participate in the Hemochromatosis and Iron Overload Screening Study (HEIRS). Your appointment has been scheduled for:

day: _____ **date**: _____ **time**: _____ : _____ am pm

Please come to: _____

A map and parking instructions are attached.
For your convenience, we have enclosed a bag for your medications.

- Please **do not** take Vitamin C or iron supplements during the two days before your visit.
- You should **fast** (take nothing by mouth except water) for 12 hours before your appointment time. However, you are encouraged to drink a glass of water before your visit. We encourage you to consume dinner at least 12 hours before your appointment. **Refreshments will be provided at your clinic visit.**

  Please begin your fast on: _____ at: _____ am
- Please **do** take the morning dose of any medicine that you normally take **except** vitamins and medications for diabetes. Do **not** take your insulin before you come to the clinic: please bring it to the clinic.
- Please **bring** all prescribed pills, dermal patches, eyedrops, creams, salves, suppositories, or injections that you have used during the two weeks prior to your appointment date, as well as all vitamins and mineral or herbal supplements that you are taking. Also, please bring any over-the-counter medicine that you have taken in the past two weeks.
- Please **complete** the enclosed Food Frequency form and Family History form prior to your appointment. Also, read the enclosed Informed Consent, but **do not** sign it. Bring this information to the clinic along with the forms. If you have difficulty completing any of the forms, please do the best you can. Clinic staff will go over these forms with you during your visit and can assist you or answer any questions you have.
- Please **refrain from smoking, vigorous exercise, and alcoholic beverages** on the morning of your visit.
- Please **bring your eyeglasses** if you need them to read.
- You may wear or bring loose fitting clothing. Please leave jewelry at home.
- The clinic visit will take approximately 2 hours. If you have any questions or a problem with your scheduled appointment time, please call the clinic at: _____

3.8 Scheduling the Comprehensive Clinical Exam Visit

Based on the results of the initial screening visit, the CoC will identify which participants are eligible for the Comprehensive Clinical Exam and provide a list to the Field Center. Clinic staff should telephone these participants to attempt to schedule them for an exam visit. If the participant indicates approval, an appointment is scheduled. An explanation of materials to be mailed (results letter, consent form and other forms) should be given. A follow-up confirmation of the appointment (See Section 3.7 above) will be mailed, along with the directions to the clinic and other materials. A follow-up call may be placed a few days before the exam to remind the participant of the clinic visit and further instructions.

Please refer to Chapter 4 for the specifics of the CCE.

4. COMPREHENSIVE CLINICAL EXAMINATION (CCE)

4.1 Overview

The HEIRS CCE will be scheduled over a three-year period, beginning February 2001. The examination will include several questionnaires, recording of medications, vitals, genetic counseling, an interview for the family study, and a physical exam. We estimate that the complete examination will require about two hours. Every effort should be made to perform all the components of the examination within an eight-week period, and preferably a four-week period, after the participant's Initial Screening visit. Clinics may vary the exam sequence to some degree, if needed, but the requirements listed below may not be altered.

a. Questionnaires, vitals measurement.

b. Blood collection should be performed while the participant is fasting. If a participant comes to the clinic non-fasting, all exam components including the venipuncture should **still** be performed. Blood pressure measurement should be done before venipuncture.

c. Blood drawing should be done after a 12-hour fast and preferably before 10:30 am., diabetics preferably before 9:00 am.

d. Counseling and interview for the family study (if appropriate).

e. Reviewing and recording of medications.

The CCE involve three types of participants: (1) those identified as C282Y homozygotes or as potential cases based on laboratory results from the Initial Screening visit, (2) those randomly selected as normal controls, and (3) family members of participants tentatively identified as having primary iron overload. The components of the exam visit for cases and controls are identical except that controls do not complete pedigrees or undergo genetic counseling. However, there are some differences for family members, and these differences are described in Section 4.9.

Data collected for the CCE will be used for several analyses, including the Case-Control Study, the Family Study, and parts of the Ethical, Legal and Social Implications (ELSI) Study. Participants completing the Initial Screening visit will be eligible for the CCE if they are C282Y homozygotes, qualify as potential cases or are selected as potential controls. The invitation to participate in the CCE will be made at the same time as results are given. Those participants who agree to attend the CCE will be mailed an Informed Consent form, a Food Frequency Questionnaire (FFQ), a Family Medical History Form, a plastic medication bag, and instructions. At the CCE, participants will be asked to complete a Medical History Form. If question # 13 is answered as "yes" (previously been told to have HH or iron overload **prior** to screening in the HEIRS study), the clinician will ask 2 additional questions about previous phlebotomy treatment and liver biopsy. Clinic staff will verify informed consent, review all forms for completeness, complete the Medications section of the Clinical Assessment Form, draw blood and perform the Physical Exam section of the Clinical Assessment Form. All

forms will be data entered locally (except the FFQ, which are stored separately and shipped monthly, see Section 4.5.5), and blood will be shipped to the Central Lab.

4.2 Recruitment for CCE

The CoC will produce a number of recruitment reports tracking the progress of the CCE phase of the study. These will be available on the website and updated frequently.

The CoC developed a website CCE Tracking system that performs many functions. Please see the Data Management Chapter 10 for specifics on the Tracking System.

4.3 Pre-Exam Instructions / Activities

Once the CCE has been scheduled, all participants should be mailed forms and the Clinic Appointment Reminder and Instruction Sheet for the visit. Be sure to verify the participant's preferred language (English, Spanish, Mandarin, Vietnamese). These materials include:

- A cover letter which each Field Center develops for its participants.
- Informed consent.
- The Food Frequency Questionnaire (note: not available in Mandarin or Vietnamese, but Vietnamese participants will have to complete this form during the visit with the assistance of a bilingual interviewer).
- The Family Pedigree (if appropriate) and Family Medical History Forms.
- A bag and instructions for bringing medications.
- Instructions for fasting.

Prior to mailing, print out a sheet of id-acrostic labels for the participant. Attach a label to each form. Keep the remaining labels in the participant's folder for later use.

Mail these Clinic Appointment Reminders and Instructions to the participant 10 to 14 days before the clinic visit and explain them over the telephone when you schedule the visit. Check the Initial Screening Checklist Form to see if the participant indicated a preference for forms in one of the translated languages. Be sure to send the form in their preferred language. If possible, make a reminder call to the participant a few days before the clinic visit and reiterate the instructions. Before the examination, make sure the participants understand the following instructions.

1. Participants should not take Vitamin C or iron supplements during the two days prior to the visit.
2. Participants must fast for at least 12 hours before the examination. This restriction applies to all food and beverages (except water), including alcohol. Instruct them to consume dinner at least 12 hours before their scheduled appointment at the clinic. Only water and prescription medications are allowed from dinner until the start of the examination the next morning. If a participant comes to the clinic non-fasting, all exam components including the venipuncture

should **still** be performed. Diabetic patients should **not** take their hypoglycemic medications the morning of the clinic visit; they should *bring the morning dose to the clinic* to be taken after venipuncture. (Diabetics need to be scheduled for the early morning appointments)

3. Participants should avoid heavy exercise during the 12 hours before the visit.
4. Participants should not smoke on the morning of the visit.
5. Participants should bring all current medications, both prescription and over-the-counter, including vitamin preparations, suppositories, and dietary supplements, to the clinic in the bag provided. If the participant forgets to bring the medications, make arrangements to obtain the medications.
6. Participants should bring the name and complete address of their personal physician or health plan, particularly if they wish to have examination results sent to that provider.
7. Participants should wear or bring loose-fitting clothes, preferably t-shirt, sweat pants, and slip-on shoes or sneakers.
8. Food Frequency Questionnaire - participant completes at home prior to CCE, however, if this is not completed, the clinic staff should instruct the participant to complete it while they are in the clinic, or the staff should assist them in completing the form.
9. Family Medical History Form - participant completes at home prior to CCE
10. Medical History Form – participant completes at the time of the CCE with the assistance of the examinor. This form is discussed with the participant during the CCE. If question # 13 is answered as "yes" (previously been told to have HH or iron overload prior to screening in the HEIRS study), the clinician will ask 2 additional questions about previous phlebotomy treatment and liver biopsy, and will data enter these questions. If the answer to #13 is no, then the 2 additional questions do not need to be asked or data entered.

Make the following preparations prior to clinic visits on days when they are scheduled:

Forms

- Collect several sets of the forms that will be completed for interviews and procedures. Pre-print labels with participant IDs for the forms, which will be matched to an individual when he/she begins the exam. Collect the set of Central Lab id labels. Forms will be printed in English, Mandarin, Vietnamese, and Spanish. For each participant, gather all the forms required for a visit, including the informed consent, medical release, and contact information form, and place into a binder labeled with his/her ID.

Supplies and Equipment

- Set up vacutainer and aliquoting tubes on the racks and attach the pre-printed labels to the tubes. Place all phlebotomy supplies on the blood drawing table. A list of supplies is available in appendix I of the CCE and Family Study Collection and Processing MOP.
- Make sure that the examination rooms are clean and have clean linen (sheets, pillow cases, wash cloth, blankets).
- Prepare the participants' gowns or (scrubs) and slippers.
- Prepare the examination room for the seated BP and physical exam. Check all instruments that will be used for the examination.
- Make sure a snack will be available for the participants.

Staffing

- Prepare staff assignment sheet and make sure everyone knows his/her responsibilities. This is particularly important if you schedule a large number of participants on a given day.

4.4 Informed Consent Overview

The first stage on arriving at the clinic should be to obtain written informed consent for the CCE phase of the study. The original informed consent does not cover this second stage. Obtaining informed consent involves two components: *assuring that people are fully informed* about this stage of the research study, and *obtaining written documentation of their agreement to participate* in this stage.

Explaining the form.

Clinic staff should be fully familiar with the contents of the consent form (Appendix E), which contains all of the required elements of consent and describes in relatively easy-to-understand language (9th grade reading level) the purpose of the CCE stage of the HEIRS study, what is expected of participants and in turn, what participants can expect from this study. *There are two different versions of the consent form - one for "cases", and the other for "controls."* Participants should have been mailed the consent form ahead of time, but many will not have read the form. If participants indicate they have read the form, then they may sign it straight away. However, it is still good practice to briefly summarize the main features. If participants have not yet read the form, they should be given time to do so. After a few minutes, check back with the participant to see if they have any questions. If there is any indication that the individual is having difficulty reading or understanding the form, the participant should be offered assistance.

Problems reading the form

Some people may have difficulty reading the consent form. Clinic staff should be aware of the average level of education of people coming into their clinics. If staff has a sense that reading is a problem, they can offer assistance in reviewing the consent form. However, it is possible that some people may take offense at the suggestion that they are unable to read, so this must be done cautiously. It is also permissible for participants to have a friend or family member assist them in reading or discussing the consent form. It is also permissible for people not to read the consent form themselves, but to listen to your explanation (which in that event should follow the details of the oral consent form). However, in all instances, the staff person should be convinced that the potential participants are competent and that they generally understand the basic elements contained in the consent form. In addition, the participant must sign the consent form. If the recruiter believes that the participant lacks the capacity to understand or competence to consent, the patient is not eligible for the study. Should this occur, the clinic visit should be discontinued or postponed in such a way that it will not offend the individual.

Non-English Speaking Participants

If potential participants are not English speaking, your records should indicate whether they asked for a translated version previously. If so, offer it again, but don't insist if it appears they prefer the English version. If communication with potential participants is not possible, those individuals should not be retained in the HEIRS study. However, it is permissible for non-English-speaking participants to rely on a friend or family member as translator in reading and understanding the consent form and other forms as long as the recruiter is comfortable with this situation.

Documentation of consent

The research participant must personally sign the consent form (or, if the participant is physically impaired but mentally competent, a legally authorized representative may sign for them). A staff person must sign the witness line. It is permissible to witness the signature even if it was signed prior to arriving in the clinic, if the participant acknowledges the signature. Signatures do not need to be notarized, and it does not matter if pencil or pen is used, although pen is much preferred. A copy or duplicate of the consent form must be given to the participant or their representative. The copy does not have to be signed. The signed and witnessed original consent form **must** be placed in the participant's study file.

Consent for Release of Medical Information

At the same time informed consent is obtained from "cases," participants must also be asked to authorize release of their medical information to the HEIRS study. There is a separate form for this purpose, which can be attached to the informed consent form or maintained separately. This form is not required for participants who are controls. "Case" participants should be told that this authorization is to allow the HEIRS study to monitor their health following their CCE. Participants have the right to refuse

permission, and can still be included in the study if they do so. For signatures and witnessing, follow the same procedures as the informed consent form. Be sure to print the subject's name and include their date of birth, for identification purposes. The original of this form **must** be kept in the participant's file. A copy does not need to be given to the participant, but they may have a copy if they ask for one.

4.5 Administration of Questionnaires and Forms

Following completion of the informed consent at the CCE, the following forms will be collected from the participant or completed by staff:

Note: Please review the forms for completeness while the participant is still in the clinic.

1. Lab Test Request Form - staff completes
2. Food Frequency Questionnaire - participant completes at home prior to CCE or is completed at the CCE with staff assistance.
3. Family Medical History Form- participant completes at home prior to CCE
4. Medical History Form- participant completes at the CCE with staff assistance.
5. Clinical Assessment Form - staff completes
6. Pedigree Form - staff completes (except for control participants)

4.5.1 Examination Order and Forms

The following guidelines identify elements of clinic order. Many elements are left to the discretion of the individual center.

1. Blood pressure could be done immediately following the greeting and informed consent, and *before* venipuncture. These components may be done in any order, but the resting blood pressure should be obtained after the subject has been in the seated position for at least five minutes.
2. Venipuncture should be performed in the fasting state after blood pressure measurement. If a participant comes to the clinic non-fasting, all exam components including the venipuncture should **still** be performed.
3. Provide a snack after completing the blood draws.
4. Questionnaires may be administered at any time during the examination. During the interviews make every effort to avoid distractions, ensure privacy, and maintain confidentiality. Do not conduct interviews during the snack or in the waiting area in the clinic.
5. Review Contact information form. Record any updates or changes on the form and local database.
6. Genetic counseling and interview for the family study (if appropriate).
7. Reviewing and recording of medications on Clinical Assessment Form.
8. Physical exam

4.5.2 Guidelines for Examination of Diabetic Participants

Diet-controlled diabetics must fast overnight and are treated the same as non-diabetics. Diabetics taking oral hypoglycemic medications or insulin must fast overnight (unless a bedtime snack was prescribed by their physician) and to come to the clinic without taking their hypoglycemic medication. They should bring their morning medication dose with them to the clinic. Schedule all known diabetics taking oral hypoglycemic medications or insulin for examination as early as possible (before 9 a.m.). Draw fasting blood samples promptly on arrival at the clinic (after measuring blood pressure). Immediately following venipuncture, serve breakfast and instruct participants to take hypoglycemic medication as prescribed.

4.5.3 Blood Pressure

Blood pressure (BP) level is a major risk factor for coronary heart disease, congestive heart failure, and stroke. Heart rate reflects autonomic nervous system function and cardiovascular fitness. The measured BP level is subject to biological and observer variability. The purpose of a specific measurement protocol, or training and certifications of technicians, and of ongoing quality control is to minimize variability due to known exogenous factors and to reduce imprecision and biases in measurement.

Materials and equipment

- Watch or stop watch (to time five-minute rest and resting heart rate).
- Clinical Assessment Form.

.

Methods

A. Preparation

Before the BP measurement procedure, explain to the participant what to expect and how long the procedure will take. The following script is suggested:

“This part of the exam involves taking your resting blood pressure. It will take about 10 minutes. We would like you to sit with both feet on the floor and your arm supported on the table. We will have you sit quietly for five minutes. Then we will take your blood pressure." Make sure the room temperature is between 70 and 76 Fahrenheit.

B. Cuff Size Selection

Use the proper cuff size to avoid under- or over-estimation of the correct blood pressure. Selection of the proper sized cuff is based on the guideline that the length of the inflatable bladder in the cuff should be at least 40% of the arm circumference**.**

C. Positioning the Participant

The workstation should be free of excessive noise or distractions.

The participant should be seated and relaxed in a comfortable chair, to ensure that:

He or she is sitting up (not slouched).

Both feet are on the floor (legs/ankles not crossed).

Right forearm is supported resting on the table.

The participant should not talk, eat, or drink during the procedure.

D. Application of the Blood Pressure Cuff

Place the appropriate cuff around the upper right arm so that the mid-height of the cuff is at heart level. Palpate the patient's brachial artery and place cuff so that the artery is aligned with the cuff arrow marked "artery."

Place the lower edge of the cuff, with its tubing connections, two centimeters above the natural crease across the inner aspect of the elbow.

Wrap the cuff snugly around the arm, with the palm of the participant's hand turned upward.

Secure the wrapped cuff firmly by applying pressure to the locking fabric fastener over the area where it is applied to the cuff.

Do not wrap the cuff too tightly around the arm. You should be able to insert the first joint of two fingers under the cuff. The cuff should be snug but not tight.

Be sure all air is squeezed out of the cuff before each inflation.

E. Rest Period

The participant should rest for five minutes (timed using a watch or stop watch) prior to the heart rate and blood pressure measurement.

F. Blood Pressure Measurement

Record the systolic and diastolic blood pressure on the *participant's* information sheet. Thank the participant for his/her time.

G. BP Measurement Instructions for Participants With Short, Thick Arms

Occasionally there will be a participant whose upper arm is too thick and short for the thigh cuff or on whom the thigh cuff pops open on inflation. The

alternative procedure in this case is to obtain the resting blood pressure in the right *forearm.*

Measure the forearm circumference at the midpoint between the olecranon and the ulnar stylus (wrist bone on pinkie side). Select the proper size cuff based on the forearm measurement. The blood pressure procedure is otherwise the same.

You must document that you have measured the *forearm blood pressure.* Write this in a *blank area* on the form.

4.5.4 Blood Draw

See Section 2.6.1 - 2.6.5 on blood collection procedures. Review the CCE Lab MOP for CCE specific procedures (Chapter 13). When the blood draw is completed, offer the participant a snack.

4.5.5 Food Frequency Questionnaire (FFQ)

Participants are sent this form prior to coming to the CCE, although some FCs have chosen to have the participant fill out the form once they have arrived for the CCE. Once the participant has finished the filling out the form, or brings it with them already filled out, please spend a few minutes (usually 2-5 minutes) to check over the answers while the participant is still in the clinic. **The goal is to identify obvious omissions or errors, and not to judge the quality of the participant's diet.**

A. Make sure the Participant ID is correct

B. Every question should have an answer. If there is no frequency information for a food item, it will not be counted even if a portion size is indicated. If any question is left blank, try to contact the subject to obtain an answer. **THE BEST THING TO DO IS THOROUGHLY REVIEW THE QUESTIONNAIRE BEFORE THE PARTICIPANT LEAVES THE CLINIC**. Check for omissions – skipped foods, missing information. Prevent skipped foods by instruction that the participant should check “Rare or Never”, rather than skipping foods they rarely or never eat. If there are any omissions, attempt to fill out the blank spaces with the participant’s help.

C. If two frequencies are checked for a dietary item, the food will not be counted, regardless of whether a portion size is indicated.

D. If no serving size is indicated, but a frequency of at least once a month is indicated, we will impute a “B” (medium) serving size. Also, if a frequency

of at least once a month is indicated and the person checks two serving sizes, we will impute a "B" serving. So the default is the "medium" portion size. Check for unlikely frequencies such as liver twice a day. If the questionnaire has mostly "1's" (1/day, 1/week, 1/month), verify that this is in fact what the participant means. . If most or all portion sizes are "medium", confirm this with the participant. Ask whether there are any foods they typically consume either very small or very large portion sizes.

E. Subjects may erase one answer and then fill in another one. Be certain the unwanted answer is completely erased.

F. Erase Stray Marks. Sometimes subjects write comments next to the answers. These will present problems if the writing is on the bubbles or on the "skunk" marks at the edges and bottom of the pages. Thus, stray marks should be erased, and the staff should take care not to make any annotations on the forms.

G. Use Pencil- The scanner cannot read answers written in ink. If a subject uses ink to fill in the bubbles, you will need to go over the responses again with pencil. We have found that hard #2 pencils are the best (and mail one to our subjects with their questionnaire). They are dark enough to get read by the scanner, yet are less likely to smear.

H. Do not fold the questionnaires. Please handle the questionnaires carefully—if they are torn, creased, wet, or smudged, the scanner will not be able to read them. If a subject returns a questionnaire in this condition, you will need to copy the responses onto a clean questionnaire before sending it to CRCH.

- Please check each form carefully, as no manual edit checks are performed at Cancer Research Center of Hawaii (CRCH).

FFQ Forms Shipping

Store the completed FFQ's separately, and mail them monthly, regardless of the quantity, to:

Shirleen Saiki
Cancer Research Center of Hawaii
1236 Lauhala St., Suite 407
Honolulu, HI 96813

We recommend that you use a courier service so the forms can be tracked if there are any shipping problems.

Do not separate the pages of the questionnaire before mailing them, because the scanner program will not work if the pages are out of order, so it is best to keep the questionnaire intact. The pages will be separated at CRCH.

4.5.6 Family History and Pedigree

See Chapter 5 on the Family Study.

4.5.7 Medical History Form

Overview

The Medical History Form (Form 5) captures information on current symptoms and signs, medical history, reproductive history for women, blood transfusion and donation, and lifestyle.

General medical history is obtained to assess the health status of the patient. The Medical History form is to be filled out for all participants at the CCE.

Completing the Medical History Form (U.S. and Canadian versions) - the participants at the CCE visit will complete the Medical History form.

Page 1. Staff will complete the header information at the top of the first page of the form. This includes participant id, acrostic, date of the visit and the interviewer code.

This form may be done in an interview format between clinician and participant.

Symptoms and Signs

Item #1-5: The participant will complete the following questions regarding having had or experiencing any of the following symptoms and signs during the past 12 months (yes, no, or don't know):

1. Swelling of feet or ankles
2. Change in skin color
3. Unexplained weight loss
4. Abdominal swelling or fluid
5. Men only: trouble having an erection or loss of sexual drive

#6-12: The participant will complete the following questions regarding being repeatedly bothered by any of the following (yes, no, or don't know):

6. Chronic fatigue/weakness
7. Shortness of breath
8. Joint stiffness/pain/ache
9. Excessive thirst
10. Polyuria (excessive urination)

11. Unexplained abdominal pain or discomfort
12. Unexplained confusion or memory loss

Medical History Information

Item #13-30: The participants will complete the following questions regarding things that may have happened or began long ago. Some of these questions may be sensitive but participants should be encouraged that this information is valuable to the study. The interviewer will answer questions if the participant does not understand questions or terminology.

These questions begin with "Has a doctor ever told you that you have or had any of the following": (answers are yes, no or don't know).

13. Iron overload or hemochromatosis

Note: Please see section 4.5.7.1 for additional items to record if this question is answered yes. Please be sure that if the answer to this question is yes, that he/she was told this **prior** to screening in the HEIRS study. If the answer to #13 is no, then the 2 additional items do not need to be asked, or data entered.

14. Anemia
15. Sickle cell anemia
16. Thalassemia or inherited anemia
17. Unusual blood loss
18. Diabetes (including more details of treatment). **Note:** This does not include gestational diabetes.
19. Liver disease (including types)
20. Thyroid disease
21. Heart failure
22. Abnormal heart rhythm, heartbeat or action
23. Other heart disease or heart attack
24. Arthritis
25. Osteoporosis
26. Porphyria cutanea tarda
27. HIV or AIDS
28. Chronic inflammation, chronic infection, autoimmune disease or lupus.

Note: “autoimmune disease” may be broadly interpreted.

29. Cancers

30. Chemotherapy or bone marrow transplant

Reproductive History (For Women Only)

Item #31-36: The participants will answer questions related to reproductive history (answers are yes, no or don't know):

31: Seen a doctor for menstrual problems, in-between bleeding, and early stopping of periods

32: Pregnancy information, including number of pregnancies and live births

33: Current pregnancy status

34: Menopause. **Note**: This can include peri-menopause

35: Age experienced menarche (first menstrual period)

36: Hysterectomy information, including age

Blood Transfusion and Donation Information

Item #37-38: The participants will answer questions related to blood transfusions and donations: (answers are yes, no or don't know)

37: Ever had blood transfusions, including number (**Note: It is extremely important to ascertain if the transfusions are less than or equal to or greater than 10 units)**

38: Ever donated whole blood at a blood bank, and number. Note: One unit is donated per visit, so the number of donations is equivalent to the number of units. The term "whole blood" is used to exclude plasma donations.

Lifestyle Information

Item #39-42: The participants will answer questions related to their lifestyle:

39: Shortness of breath when resting, walking on level ground or walking quickly

40: Alcoholic beverage consumption

41: Age when began consuming alcoholic beverages

42: Present status of alcoholic beverage consumption

Demographics

Item #43-47: The participants will answer questions related to their education:

43: Level of education

NOTE: Questions 44-47 apply only to non-clinical participants (family members). These questions are related to gender, birth date, Hispanic, Latino, or Spanish descent, and racial category.

44: Gender

45: Birth date

46: Spanish, Latino, or Hispanic descent

47: Broad racial category. Note: Copy from instructions for question #4 on “Initial Screening Form”.

4.5.7.1. Additional Items to be included with the Medical History Form

There are two additional items that need to be reported at the CCE, but only if the participant answered yes to # 13 of the Medical History form. These two items are on a separate sheet, but go with the Medical History Form. If the participant answered "yes" to question # 13 (have you ever been told you had iron overload or hereditary hemochromatosis **prior** to screening for the HEIRS study), the following two questions should be asked and the responses recorded:

1). Have you ever had phlebotomy as treatment for your iron overload or HH? The interviewer must be certain to distinguish between blood donation and treatment phlebotomy. The answer is yes or no.

2). Have you ever had a liver biopsy? The answer is yes or no.

End of form

4.5.8 Clinical Assessment Form (Form 6) and Procedures

At the CCE, a Clinical Assessment, including medications reception and physical exam, will be obtained for each participant.

Overview

Persons included in the Clinical Assessment. Clinical assessments will be performed on participants who, on the basis of the initial screen, are found to be (1) C282Y homozygotes, (2) other participants whose screening TS and SF values exceed both cutoffs in Table 1 below, (3) control participants whose screening TS and SF values fall in the range in Table 2 below, or (4) family members of certain C282Y homozygotes and certain other participants who meet the criteria for probably having primary iron overload.

Table 1. Cutoffs for TS and SF

	Males	Females
TS	50	45
SF	300	200

Table 2. Eligible ranges for potential controls

	Males	Females
TS	20-34	16-28
SF	87-247	29-121

Purpose of the Clinical Assessment. The purposes of the clinical assessment are (1) to determine if a participant has primary iron overload versus secondary iron overload, inflammation, or normal iron status and (2) to determine the clinical consequences of C282Y homozygotes and other forms of primary iron overload.

Specific Procedures for the Clinical Assessment

Medication Reception

HEIRS will describe all medications that participants are using, both prescription and over-the-counter (OTC). These medications will include pills, suppositories, liquid medications, skin patches, eye drops, creams, salves, inhalers and injections, as well as cold or allergy medications, vitamins, herbal remedies, and other supplements. After the Initial Screening visit, participants will receive an appointment/instruction letter regarding which medications are to be brought to the CCE visit and a bag will be provided to contain these medications.

Information regarding medications will be recorded on the Clinical Assessment form by the clinic staff, Part 1. At the CCE visit, the clinic staff will review with each participant the prescription and OTC medications taken within the last two weeks and examine the

medications' containers and record medications taken by the participant. Some participants may not have taken any medications in the past 2 weeks. If the medications had been taken and were not brought to the visit, arrangements should be made to obtain the medications. Participants may refuse to bring the medications in, but every attempt to obtain this information should be made. If the participant has not brought medications, but has a card or other list, this is acceptable, but not preferred.

Completing the Medications Reception (Form 6: pages 1 and 2)

Medications information will be recorded in Part 1 of the Clinical Assessment Form. Clinic staff will complete the identifying information at the top of the form for participant id, acrostic, date of visit, and completer code.

Part 1: Questions 1-6:

1. Clinic staff will verify if medications taken in the last 2 weeks were brought to the clinic. (answers are yes and no) If medications were brought, check the appropriate box on the form. If medications were not brought, check the box indicating that medications were not brought in and arrangements should be made to obtain them.
2. The clinic staff should inquire if the medications brought to the clinic were all of the medications that were taken in the past 2 weeks. If so, the appropriate box should be checked. If not all medications were brought in, the appropriate response should be checked - arrangements to obtain were made, or the participant took no medications, or participant refused to bring in medications. If the participant refused to bring in medications, staff should inquire and record the reason in the comment field.
3. Prescription medication names (first 20 letters of the medication name) are to be recorded in the prescription medications section of the form. Note that the generic name, not the trade name, should be used. This information should be legible for data entry. Eight medications may be listed in this area on the form. If more than 8 medications have been brought in, the clinic staff should prioritize the medications to be recorded. "Priority should be given to those medications which are (or are suspected to be) used for complications of hemochromatosis and iron overload." The list below includes complications of hemochromatosis and iron overload plus related conditions that we would like to confirm:
    - Liver disease
    - Diabetes (oral hypoglycemics, insulin)
    - Heart disease (antihypertensives, antiarrhythmic agents, medication for edema or CHS)
    - Arthritis
    - Menopause or osteoporosis
    - Anemia
    - Thyroid disease
    - Autoimmune diseases

If there are too many medications related to these conditions, begin with those related to conditions at the beginning of the list.

4. If medications were unable to be transcribed due to lack of space, the clinic staff should enter the number of untranscribable medications.

5. Clinic staff will verify any over the counter (OTC) medications, if any were taken in the last 2 weeks that were brought to the clinic. OTC medication names (first 20 letters of the OTC medications) are to be recorded in the OTC medications section of the form. This information should be legible for data entry. Five medications may be listed in this area on the form. If more than 5 medications have been brought in, the clinic staff should prioritize the medications to be recorded. "Priority should be given to those medications which are (or are suspected to be) used for complications of hemochromatosis and iron overload". The list below includes complications of hemochromatosis and iron overload plus related conditions that we would like to confirm:

    Liver disease
    Diabetes (oral hypoglycemics, insulin)
    Heart disease (antihypertensives, antiarrhythmic agents, medication for edema or CHS)
    Arthritis
    Menopause or osteoporosis
    Anemia
    Thyroid disease
    Autoimmune diseases

    If there are too many medications related to these conditions, begin with those related to conditions at the beginning of the list.

6. If medications were unable to be transcribed, the clinic staff should enter the number of untranscribable medications.

After reviewing and recording medication information, the medications are to be returned to the medication bag and given back to the participant.

Immediately following the listing of the OTC Medications, there is a place to put the Fairview lab id label. This is the lab id from the blood draw which is also placed on the Lab Test Request Form. There is also a place to record the "Fairview Replicate", which is the quality control blind replicate lab id number. This should only be used for the blind replicate blood specmens.

<u>Physical Exam</u> (Form 6: page 3)

Begin by reviewing the Medical History Form with the participant. There may be questions as to some of the items on this form which need to be clarified.

Determine the participant's height, weight, pulse, systolic and diastolic blood pressures, and temperature. Enter the results in Part 2 of the HEIRS Clinical Assessment Form entitled "Physical Exam," items 7 to 12.

Part 2: Questions 7-19:

Questions 7 & 8: General Instructions for Body Size Measurements

For all measurements, participants should wear light clothing but no shoes. Keep a supply of surgical scrubs at the clinical center for participants who forget to wear or bring the appropriate clothes. Have participants completely empty their pockets and remove excessive amounts of jewelry that could affect the weight measurement. Provide lockers with locks for valuables.

Take a single measurement at each body site and record using specific rounding rules. Record any modifications in measurement techniques (e.g., height decreased from a hunched posture or weight that exceeds the capacity of the scale) on the Clinical Assessment Form.

7. Standing Body Height

   Before measuring height, check to make sure the floor is level, the wall is at a 90 degree angle to the floor, the wall is straight, and the Stadiometer is mounted perpendicular to the floor.

   For accurate measurement of height, the participant must be standing in a vertical plane. To achieve this position, have the participant stand erect on the floor or horizontal platform, with back against the vertical Stadiometer, heels against the wall, and feet *or* knees together—whichever come together first. Have the participant look straight ahead, with head in the Frankfort horizontal plane.

   Place the headboard over the crown of the head, with the headboard forming a right angle to the scale. The headboard should touch the scalp lightly.

   Ask the participant to step out from under the headboard. *Record the participant's height on the Clinical Assessment Form.*

   If you are unable to measure the actual height of the participant because the headboard does not rest directly over the scalp, *estimate height.*

   Do not attempt to measure height if the participants can't stand or is in a wheelchair.

8. <u>Body Weight</u>

   Balance the scale so that the indicator is at zero when no weight is on the scale. The scale should be on a firm, level surface (not on a carpet, for example). Instruct the participant to stand in the middle of the platform of the balance scale, with head erect and eyes looking straight ahead. Adjust the weight on the indicator until it is balanced. *Record the results, on the Clinical Assessment Form.*

If the participant is too obese to stand securely on the scale's platform when looking straight ahead, he/she may stand sideways on the scale to take the weight measurement; facing to the side rather than the front will provide the participant a wider base and more stability.

If a participant has a prosthetic limb or breast prosthesis, measure weight with the limb *on.*

If a participant is frail or unsteady, measure weight while participant is lightly steadied by you or an assistant.

If a participant is unable to stand on the scale for a weight measurement, do not attempt a weight measurement.

Questions 9-12: Temperature and pulse: self-explanatory. Systolic and diastolic blood pressure: see Section 4.5.3.

Additional Physical Examinations

Questions 13-19: Perform a limited physical examination directed to the liver, spleen, heart, skin and metacarpophalangeal joints. Fill out Part 2 of the HEIRS Clinical Assessment Form entitled “Physical Exam”, questions 13 to 19, checking only one of the options of Yes, No or Not sure for each item or sub-item:

Questions 13-19: Perform a limited physical examination directed to the liver, spleen, heart, skin and metacarpophalangeal joints. Fill out Part 2 of the HEIRS Clinical Assessment Form entitled “Physical Exam”, questions 13 to 19, checking only one of the options of Yes, No or Not sure for each item or sub-item:

13. Hepatomegaly is defined as the liver being palpable 2 or more cm below the right costal margin in the mid-clavicular line or 2 or more cm below the xiphoid process in the midline.
14. Splenomegaly is defined as the spleen being palpable to any extent below the left costal margin.
15. Listen to the heart sounds with a stethoscope. Take the pulse for at least one minute. Bradycardia is defined as less than 40 beats per minute. Tachycardia is defined as more than 100 beats per minute. Frequent premature contractions are defined as one or more ectopic beats per minute.
16. Murmur is defined as a prolonged sound from the heart occurring either in systole or diastole.
17. Edema is defined as symmetrical swelling of dependent areas such as the lower legs or the presacral area.
18. Increased pigmentation is defined as generalized augmented shades of gray or brown on sun-exposed or unexposed areas of the skin.
19. Blistering, ulcerations and scarring of the sun-exposed skin can be evidence of porphyria cutanea tarda.

20. Hypertrichosis is defined as excess hair growth on the sun-exposed skin, especially on the face lateral to the eyes. Hypertrichosis can be evidence of porphyria cutanea tarda.
21. Swelling or tenderness of the metacarpophalangeal (MP) joints, especially the first and second MP joints, can be found in HFE Hemochromatosis.

End of form

4.6 Forms Review and Data Entry

Clinic staff should ensure that forms are processed in a timely fashion, from data collection to data entry and final storage of forms. Please refer to Chapter 10 for expanded data entry instructions.

1. As stated in the Initial Screening Chapter, clinic staff should review the forms while the participant is in the clinic, possibly rather than by a subsequent telephone call to retrieve or clarify responses or items that were left blank. A clarification for a blank or inappropriate response is easier to correct or obtain while the participant is in the clinic rather than a subsequent follow-up telephone call. Some questions may been sensitive and intimidating to a participant; therefore, the participant may have intentionally left some questions unanswered. Use discretion in trying to obtain further information from the participant. It is important that we respect the participant's right to refuse to answer any questions that he/she chooses.
2. At the CCE visit, staff may want to review any forms that were completed at home by the participant while/or after the snack is being presented after the participant has had the blood draw.
3. All forms for a participant should remain together. Upon completion and review of the forms at the CCE visit, staff should place all the forms completed for each participant, including the Lab Request form, in a folder (labeled with the participant's identifying information, such as name, acrostic, or identification number) to ensure that forms will not be lost or misplaced prior to data entry. The FFQ can be stored separately, along with all of the FFQs that will be sent in monthly.
4. After all forms have been reviewed, staff should place the forms back in the participant's folder and forward to the data entry staff for entry in the computer. After entry, the folder containing the forms should be filed appropriately according to the clinic's discretion (alphabetical, numerically, etc.) for easy retrieval and filing of additional forms at a later date.

4.7 Form 7 Clinical Exam Checklist

Overview

The Clinical Exam Checklist (Form 7) serves as a summary for procedures and forms performed and collected at the CCE visit and genetic counseling. Any significant findings related to the CCE, including laboratory findings, are captured on this form. The Clinical Exam Checklist is to be filled out for all participants at the CCE. This information will not be entered in the database.

Completing the Clinical Exam Checklist - the staff will complete the Clinical Exam Checklist after all procedures in Part 1 - Comprehensive Exam Visit have been performed. The genetic counseling section may be done at this time. The remainder of the form, Part 2 - Laboratory, will be completed after results have been received and information is available.

Part 1: Comprehensive Exam Visit

Questions 1-8: The staff will complete the following questions regarding the CCE visit (the answers are yes, no):

1. Informed consent obtained, including level of consent
2. Medical History obtained
3. Family History obtained
4. Food Frequency Questionnaire obtained
5. Medications recorded
6. Physical Exam performed
7. Blood obtained
8. Clinically significant conditions found

Note: **If <u>Yes</u>: Answer additional questions (what was significant, referral to physician for follow-up, name of physician, referred by, and date of referral, etc)**

If <u>No</u>: Go to question 9.

Part 2: Laboratory Findings and Genetic Counseling

(Please refer to chapter 6 for genetic counseling guidelines.)

Questions 9a-v and 10: The staff will complete the following questions regarding laboratory findings after lab results are obtained:

Were any clinically significant lab results found (yes or no)

9a. If yes, list significant finding

9b. Were lab results provided to participant (yes or no)

Check Normal or Abnormal for the following tests results:

9c. TS

9d. SF

9e. DNA

9f. CBC

9g. Glucose

9h. Insulin

9i. ALT

9j. AST

9k. GGT

9l. CRP

9m. Hep B surface antigen

9n. Hep C antibody

9o. Reticulocyte

9p. Haptoglobin

9q. LDH

9r. Indirect bilirubin

9s. Was participant referred for follow-up (yes,no)

9t. Name of physician

9u. Referred by (name)

9v. Date of referral

10. Was genetic counseling provided (yes, no)

Note: Please refer to Chapter 6 for specifics of genetic counseling.

10a. If yes, list name of genetic counselor

10b. Was participant invited to participate in the Family Study (yes,no)?

End of form

After completion of Part 1, file the Clinical Exam Checklist in the participant's chart. Upon receipt of the lab results, record the findings in Part 2 of the form. After completing the entire form, file the Clinical Exam Checklist in the participant's chart.

4.8 Results of the CCE

4.8.1 Results and Alert Values

Some participants who receive the CCE will have repeat values for TS and SF that are outside the typical range for a healthy individual. In this case, the participant may have

a clinical condition that should be evaluated by a physician. The table below gives the low and the high thresholds for each laboratory test and each gender.

Laboratory Test	Gender	Low	High
Transferrin Saturation	Men	15%	50%
	Women	15%	50%
Serum Ferritin Concentration	Men	25 ng/mL	530 ng/mL
	Women	15 ng/mL	300 ng/mL

Note that the procedures for individual Field Centers procedures may vary regarding whether telephone calls will be made and/or letters will be sent.

4.8.2 Additional Lab Analyses on Stored Blood

See Chapter 13 on Lab Procedures

4.8.3 Identification of potential primary iron overload cases

Cases will include:

a. All C282Y homozygotes, whatever their iron status, and
b. All other genotypes who have either a provisional classification of primary iron overload or documented primary iron overload.

The CoC will identify the participants that are C282Y homozygotes as well as the provisional iron overload cases. These are posted on the HEIRS website under the "Data" menu, then "Tracking System." The report is called "Quantitative Phlebotomy/Liver Biopsy Follow-up." Each site will have a site-specific list, which will include the participant ids, acrostics, date of IS and date of CCE for all participants that should be followed for QPh and/or liver biopsy.
Note: Please refer to Chapter 8 -Quantitative Phlebotomy-for more specific details.

Provisional classification of primary iron overload. The provisional classification of primary iron overload will be based on having confirmed elevations of both serum ferritin concentration (> 200 ng/ml women; > 300 ng/ml men) and transferrin saturation (> 45% women; >50% men), and no evidence of:

a) elevated SF due to inflammation (evidence of inflammation will include inflammation present by history, and/or elevated CRP) or
b) elevated serum ferritin concentration and transferrin saturation due to hepatocellular dysfunction (evidence of hepatocellular dysfunction will include elevations in ALT and/or AST) or
c) secondary IO (evidence of secondary IO will include lifetime history of blood transfusions more than 10 units and/or anemia).

Specifically, for a participant who presents with both elevated serum ferritin concentration and transferrin saturation, there are four questions to ask. If the answer to each question is no, then the subject will be provisionally classified as having primary iron overload. If there are yes answers, the subject may need further evaluation to document whether or not there is primary iron overload. See the next section for further discussion.

1. Is inflammation present by history (e.g. active cancer, SLE, other connective tissue disease, active or chronic infection) or C-reactive protein (> 2 mg/dl)?

Participants in the 'Yes' category will include those answering 'Yes' to **any** of Q19c (liver cancer), Q28 (chronic inflammation, chronic infection, autoimmune disease or lupus), Q30 (chemotherapy or bone marrow transplant) on the Medical History Form **or** having lab results with a CRP value greater than 2 mg/dl.

2. Is hepatocellular dysfunction present (ALT > 60 IU/L or AST > 60 IU/L)?

Participants in the 'Yes' category will tentatively include those having lab results with ALT greater than 60 IU/L or AST > 60 IU/L. Hepatitis testing will be done for participants having ALT >40 IU/L for males and > 31 IU/L for females. **If ALT is 1.0 times the upper limit of the normal reference range (which will be indicated in the lab data), tests for hepatitis B surface antigen and hepatitis C antibody will be done.** If these additional tests are negative, the participant is considered to be in the 'No' category.

3. Is lifetime history of blood transfusions (not given for hemorrhage) more than 10 units?

Participants in the 'Yes' category will include those answering greater than 10 units to Q38a (how many lifetime pints/units during blood transfusions) on the Medical History Form.

4. Is anemia present (hemoglobin concentration < 13 g/dL in men or 11 g/dL in women)?

Participants in the 'Yes' category will tentatively include those having lab results for hemoglobin (part of the CBC) indicated. **For all participants tentatively in the 'Yes' category, the following further tests will be done: reticulocyte count, hemoglobin identification, hemoglobin A2 quantification, haptoglobin, LDH, and bilirubin (total, direct and indirect).** If these additional tests are all within the normal range, the participant will be considered to be in the 'No' category.

The subjects who fulfill the criteria for provisional classification of iron overload by "no" answers to these four questions will then be referred for either quantitative phlebotomy therapy or diagnostic liver biopsy. Further evaluation and treatment will not be a funded part of the study but concerted efforts will be made to capture this information. The initial assessment of prevalence of primary iron overload in this study will be based on the provisional classification.

Documented iron overload. Efforts to document primary iron overload will not be funded by the study, but concerted efforts will be made to capture this information. The following groups of patients will be referred for further evaluation and possible documentation of primary iron overload:

a) C282Y homozygotes with confirmed elevations in both serum ferritin concentration and transferrin saturation as described above,

b) subjects who fulfill the criteria for the provisional classification of primary iron overload as described in the preceding section, and

c) certain subjects with confirmed elevations in both serum ferritin concentration and transferrin saturation but who do not fulfill the criteria for the provisional classification.

For subjects fitting into categories a and b, we will recommend to the physician in charge of further management that the subject undergo either quantitative phlebotomy or diagnostic liver biopsy. In the case of C282Y homozygotes, we will recommend quantitative phlebotomy over liver biopsy if the serum ferritin is less than 1000 ng/ml and the liver function tests are normal. Otherwise, the approach will be up to the discretion of the physician.

For subjects fitting into category c, the recommendations to the physician in charge of further management will be more complex.

1. Inflammation. If the patient with confirmed elevations in both serum ferritin concentration and transferrin saturation has an inflammatory process that is temporary (musculoskeletal injury, recent surgery, acute and treatable infection), then we will recommend to the physician in charge of on-going medical care that the serum ferritin concentration and transferrin saturation be repeated in 1-2 months. If the serum ferritin concentration falls below the study threshold at this time, the patient will be classified as not having primary iron overload and we will not try to collect further information. If the patient's inflammatory process is chronic, or the elevations in both serum ferritin concentration and transferrin saturation persist after the inflammation has subsided, then we will recommend to the physician in charge of on-going medical care that the patient undergo either quantitative phlebotomy therapy or diagnostic liver biopsy, and we will make concerted efforts to collect the results.

2. Liver dysfunction. If the subject with confirmed elevations in both serum ferritin concentration and transferrin saturation also has elevated liver function tests, then we will recommend to the physician in charge of ongoing care that the patient undergo either quantitative phlebotomy therapy or diagnostic liver biopsy. The physician in charge of ongoing care will no doubt also wish to do a further workup for hepatic disease, which may include history of use of alcohol and other drugs or

medications; testing for hepatitis B surface antigen or antibody to hepatitis C; testing for Wilson's disease, alpha-1 antitrypsin deficiency, auto-immune hepatitis, sclerosing cholangitis, metastaes, infection.

3. Blood transfusions. If the subject with confirmed elevations in both serum ferritin concentration and transferrin saturation has a history of receiving more than 10 units of blood not for hemorrhage, then we will consider this subject as a case of secondary iron overload and we will not try to collect further information.

4. Anemia.

Anemia and ineffective erythropoiesis. If the subject with confirmed elevations in both serum ferritin concentration and transferrin saturation has anemia and evidence for ineffective erythropoiesis after the CCE, then we will consider this subject to be a case of secondary iron overload and we will not try to collect further information. Evidence for ineffective erythropoiesis will be based on elevation in LDH and indirect bilirubin, decrease in haptoglobin and no increase in reticulocytes. Results from hemoglobin electrophoresis and quantitative determinations of A2 and F will also be helpful in characterizing these subjects.

Anemia and inflammation. These subjects with confirmed elevations in both serum ferritin concentration and transferrin saturation will be approached as described in point no. 1 of this section.

Anemia and bone marrow suppression. Subjects with confirmed elevations in both serum ferritin concentration and transferrin saturation will fit into this category if they have anemia with normal reticulocytes, indirect bilirubin, LDH and haptoglobin and history of chemotherapy, radiation therapy, aplastic anemia, pure red cell aplasia, or myelodysplasia. These subjects will be classified as probably having secondary iron overload (shift from red cell compartment), but we will recommend to the physician in charge of ongoing care to repeat the serum ferritin concentration and transferrin saturation should the bone marrow suppression be relieved. We will recommend that those subjects with persisting elevations in both serum ferritin concentration and transferrin saturation three months after bone marrow suppression has resolved to undergo quantitative phlebotomy or diagnostic liver biopsy.

Hemolytic anemia. Subjects with confirmed elevations in both serum ferritin concentration and transferrin saturation will fit into this category if they have anemia with elevated reticulocytes, indirect bilirubin and LDH and low haptoglobin. We will recommend to the physician in charge of ongoing care that the serum ferritin concentration and transferrin saturation be repeated three months after the hemolysis has resolved. For patients with persisting elevations in both serum ferritin concentration and transferrin saturation at this time, we will recommend either quantitative phlebotomy or diagnostic liver biopsy.

Unspecified anemia. For subjects with confirmed elevations in both serum ferritin concentration and transferrin saturation and anemia that does not fit into the above categories, we will recommend to the physician in charge of ongoing care that the patient undergo either quantitative phlebotomy therapy of diagnostic liver biopsy.

Interpretation of results of quantitative phlebotomy and diagnostic liver biopsy.

Amount of iron	Hepatic iron	Interpretation

removed at quantitative phlebotomy (grams)	concentration in liver biopsy specimen (micromoles Fe/gram dry weight)	
0.2-2	< 30	Normal iron status
2-4	30-90	Mild iron overload
4-6	90-180	Moderate iron overload
>6	>180	Substantial iron overload
>10	>360	Severe iron overload

Any subject with normal iron status will not be considered to have primary iron overload.

Excluding subjects with ineffective erythropoiesis or blood transfusions:

-Any subject with moderate grade of iron overload or higher will be considered to have primary iron overload.

-Subjects with mild iron overload, normal hepatic function, and no anemia will be considered to have primary iron overload.

-Subjects with mild iron overload along with abnormal hepatic dysfunction or anemia will be evaluated by an adjudication committee to decide if they have primary iron overload.

4.9 The CCE for Family Members

The CCE for participants in the family study differ in three areas:

1. The blood draw and lab tests include an HFE screen,
2. They complete some additional questions at the end of the Medical History Form,
3. The pedigree collection and genetic counseling.

Family members will be mailed the Family History Form, but pedigree information will be collected only if there are inconsistencies with the proband's pedigree.

Note: Please see Chapter 5-Family Study-for more specifics on the Family Study.

5. FAMILY STUDY

5.1 Overview

The HEIRS Family Study is designed to identify probands from among comprehensive clinical exam (CCE) examinees, and to enroll approximately 2,000 relatives of probands for participation in the CCE. CCE participants who are not relatives of a proband will be eligible to be probands if they meet the Family Study Eligibility Criteria (described in Section 5.3 below).

- The Initial Screening Visit will result in approximately 2000 subjects eligible and willing to undergo the CCE. Once the Field Center contacts the participant and schedules the CCE visit, the Initial Screening Checklist should be checked to see if the participant indicated a language preference for forms in one of the translated languages. Then, the CCE forms will be mailed to the participant, and include:
  - a consent form, a Food Frequency Questionnaire, a plastic medication bag, instructions for the CCE, directions to the clinic, a Family History form, the result of the screening tests and an appointment confirmation letter. They will be asked to read the consent form, complete the Family History form and Food Frequency Questionnaire, and bring these completed forms, as well as their medications, to the CCE visit. **(See Chapter 4 for complete CCE procedures)**
  - At the time of the CCE visit the information contained in the Family History form will be reviewed and clarified by an interviewer/genetic counselor or another trained health professional. A pedigree will be constructed on the Pedigree form from the information provided and reviewed with the study participant. The health care provider conducting the CCE and the person conducting the genetic counseling will also use the family history of disease and pedigree information.
  - If after an initial assessment of the family structure, the family of a participant appears that it may meet the criteria for inclusion in the family study, the Family Eligibility form will be completed.
  - Utilizing the Family Contact form, contact information will be obtained from the proband on those family members for whom permission to contact has been given.
  - Following completion of the latter form and when the results of the CCE are known, which may require waiting until the laboratory results are available, a final decision will be made as to whether the participant's family meets the family study criteria. Eligibility of probands and families must be confirmed by the CoC before family member CCEs are initiated by the FC.
  - It is estimated that the families of 290 probands will be eligible for participation in the family study. Those probands who are C282Y/C282Y and who also meet the family study criteria (see eligibility criteria in Section 5.3) will be asked, before they leave the CCE visit, for permission to contact the family members, and to contact each family member and alert them that they may be contacted by the FC staff and asked to participate in the family study. The probands who are

determined to be provisional iron overload cases after the CCE results are known, and who also meet the family study criteria, will then be asked to contact family members about the family study, give permission to contact, and alert them that the FC staff may call and ask them to participate in the family study. The Reminder to Proband to contact their family members will be given to the C282Y/C282Y proband, who also meets family study criteria, before leaving the CCE visit. The Reminder to Proband to contact their family members will be given to the non-C282Y/C282Y proband who meets family study criteria upon completion of the CCE results, when it is known if the proband meets the provisional iron overload case status.

- The method of contacting family members and completing the Family Contact Form by probands and/or family members will vary across Field Centers. The proband will be asked if the FC can contact their family members at least nineteen years of age or older by use of the Family Letter from FC. If the FC or proband prefers the Letter from Proband, it can be generated either before the proband leaves the CCE or at a later time when the proband has been found eligible for participation in the family study. Ideally the Letter from Proband will be signed by the proband and sent to the proband's family member by the FC.
-

After sufficient time (typically 1-2 weeks) has passed to allow the proband's family members to receive information concerning the study from the proband and/or FC, the family member will be called by a staff person from the FC and asked to participate in the Family Study. The Phone Script for Family will be used as a template with each FC modifying according to local needs. After permission to participate in the Family Study has been obtained from a family member, they will be sent the Family History of Disease form and asked to bring the completed form with them to the CCE visit.

- The Family History of Disease information will be reviewed and clarified by an interviewer/genetic counselor or another trained health professional. The information will be cross-checked with the information provided by the proband and the pedigree previously drawn. If the family member provides information on themselves that differs from that provided by the proband, the pedigree will be revised accordingly. The health care provider conducting the CCE and the person conducting the genetic counseling will also use the Family History of Disease and Pedigree information.

5.2 Definitions

Full siblings - full siblings share a biologic mother and father.
Half siblings - half siblings share either a biologic mother or father, but not both.
Stepsiblings - stepsiblings share neither a biologic mother nor father.

Among these three, only individuals with full sibling relationships are counted toward the minimum pedigree requirements (see eligibility criteria below). Half

siblings will be eligible for participation as family members. Stepsiblings will not be eligible for participation as family members.

5.3 Family Study Eligibility Criteria

Potential probands for the HEIRS Family study will be chosen at the time of the CCE for C282Y homozygotes and after the CCE lab results are reported for the non C282Y homozygotes to ensure the provisional case status. Eligibility of each proband and family will be confirmed by the CoC before family member CCEs are initiated by the FC. Eligibility criteria are based on either the genotype or phenotype of the proband and their available family size and structure.

CCE participants will be eligible to be probands if

(1) They meet either (a) or (b):
   (a) Have the C282Y/C282Y genotype;
   (b) Have HEIRS Study criteria for provisional iron overload, regardless of genotype;

and

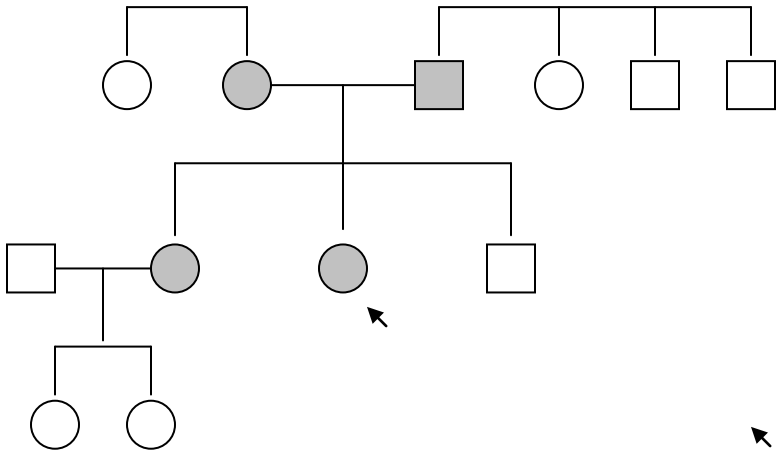
(2) They have family members, at least 19 years of age and the proband deems them available for study, which include a group of at least one of the following:
   (a) Four first degree relatives of the proband (first degree relatives are biologic parents, full siblings, and offspring);
   (b) Three offspring of the proband by the same mating;
   (c) Two full siblings of the proband.

   For a proband and family to be eligible for the Family Study, the proband must confirm that within the family, the members who are potentially interested and available to participate in the Family Study include a group that meets at least one of these three structures, (a), (b), or (c). Previously, these family members have been referred

In the case of (1)(a), families will be used to map modifier genes, and in the case of (1)(b), families will be used to map new genes for iron overload. In each case, the pedigree structures are the minimal criteria for families. An effort will be made to recruit large families (optimal family structures) to increase the power to detect new genes (note: current budget only allows for 2000 family CCEs - we need to lower the number of probands to accommodate larger families). Optimal family structures consist of 1) a nuclear family of at least four age-eligible individuals (including the proband), of whom at least one is a parent; and 2) at least eight additional age-eligible first-degree relatives of nuclear family members. (Note: first-degree relatives include parents, children, and full siblings). To meet the optimal family structure, all of the above individuals must

be potentially interested and available to participate in the Family Study as reported by the proband. Spouses/partners should be included in the Family Study if they are parents of at least one eligible family member. Siblings, parents and more extended biologic relatives of the proband's spouse/partner should generally receive lower priority than biologic relatives of the proband for participation in the Family Study.

An example of the optimal family structure is:



The shaded symbols above represent a nuclear family of size four (two parents and two children). The unfilled symbols represent the eight first-degree relatives. Four are full siblings of the parents, one is a full sibling of the children, one is a spouse of one child and they have two children who also contribute to the total count of eight first-degree relatives of the nuclear family. All individuals in this pedigree are age eligible (19 years of age or older) and are potentially interested and able to participate in the Family Study as reported by the proband.

The Family Study Subcommittee will review the structures of families targeted for enrollment and will consider changes to the above criteria if warranted after the first six months of the Family Study. A hardcopy duplicate of each Family Study proband's Pedigree Form will be faxed or mailed to Leora Henkin at the CoC upon data entry (see section 5.7.4 below).

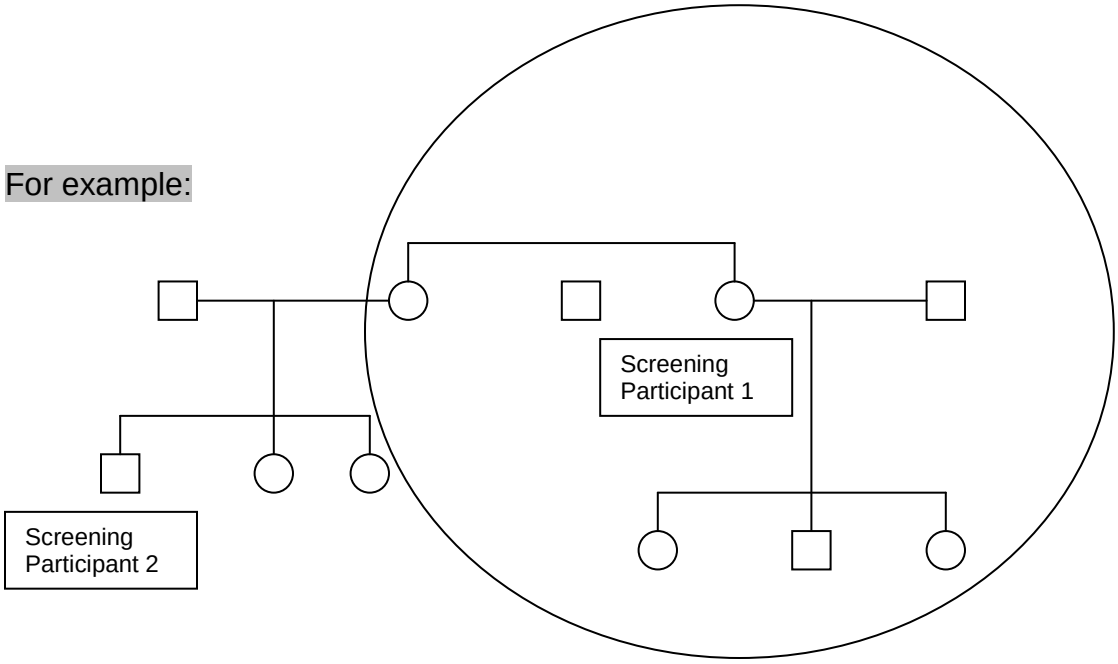
5.4 Family Study Exclusion Criteria

- Individuals 18 years of age and younger are excluded from being family member participants.

- Potential probands can be excluded from the HEIRS family study if it is determined that they have secondary iron overload after the CCE.

- A known biological relative of a proband is excluded from being a proband. However, this person and her/his relatives may be included as part of the relevant proband's larger family. If any questions arise about these exclusion criteria, please consult with Beverly Mellen Snively at the CoC for assistance.

For example:



Suppose participant 1's close family meets the optional family structure criterion (circled) and screening participant 2's close family also meets family study criteria. If participant 1 were studied first, she would have been

designated as a proband for the family study. Participant 2 cannot be a proband for the family study, but he and his family members may be included in the family study as part of participant 1's family.

If participant 2's family does not meet minimum criteria, he and his family similarly may be included as part of participant 2's family.

5.5 Recruitment and Retention Strategies

5.5.1 Probands

The overall goal will be to recruit families who live locally and who meet the pedigree structure requirements as outlined above. Families of HEIRS probands will be prioritized based on pedigree structure and potential availability of family members as reported by probands.

5.5.2 Family Members

Efforts will be made to maximize individual participation and to encourage relatives to participate (within the budget limitations of each site). These efforts include financial incentives; flexible scheduling, including Saturday clinics and travel support. Non-local family members will be encouraged to schedule a CCE when they travel to town to visit one of their relatives. If a family member lives near another FC, arrangements can be made to perform the CCE there.

5.6 Obtaining Informed Consent

The first stage on arriving at the clinic should be to obtain written informed consent for the family study. This is a critical component of this study and sufficient time must be provided in order for adequate informed consent to be secured. Obtaining informed consent involves two components; *assuring that people are fully informed* about this stage of the research study, and *obtaining written documentation of their agreement to participate* in this stage.

Explaining the form.

Clinic staff should be fully familiar with the contents of the consent form (Appendix E), which contains all of the required elements of consent and describes in relatively easy-to-understand language (9^{th} grade reading level) the purpose of the family study, what is expected of participants and in turn, what participants can expect from this study. Participants should have been mailed the consent form ahead of time, but many will not have read the form. If participants indicate they have read the form, then they may sign it straight away. However, it is still good practice to briefly summarize the main features. If participants have not yet read the form, they should be given time to do so. After a few minutes, check back with the participant to see if they have any questions. If

there is any indication that the individual is having difficulty reading or understanding the form, the participant should be offered assistance.

Problems reading the form.

Some people may have difficulty reading the consent form. Clinic staff should be aware of the average level of education of people coming into their clinics. If staff have a sense that reading is a problem, they can offer assistance in reviewing the consent form. However, it is possible that some people may take offense at the suggestion that they are unable to read, so this must be done cautiously. It is also permissible for participants to have a friend or family member assist them in reading or discussing the consent form. It is also permissible for people not to read the consent form themselves, but to listen to your explanation (which in that event should follow the details of the oral consent form). However, in all instances, the staff person should be convinced that the potential participants are competent and that they generally understand the basic elements contained in the consent form. In addition, the participant must sign the consent form. If the recruiter believes that the participant lacks the capacity to understand or competence to consent, the patient is not eligible for the study. Should this occur, the clinic visit should be discontinued or postponed in such a way that it will not offend the individual.

Non-English Speaking Participants:

In some clinics, potential participants will not be English-speaking. Consent forms will be translated into Spanish, Vietnamese, and Mandarin, but not other languages. If communication with potential participants is not possible, those individuals should not be recruited into the HEIRS family study. If there is any indication that English is not the potential participant's first language, recruiters should ascertain whether another language is preferred. It is permissible for non-English-speaking participants to rely on a friend or family member as translator in reading and understanding the consent form and other forms as long as the recruiter is comfortable with this situation.

Documentation of consent:

The research participant must personally sign the consent form (or, if the participant is physically impaired but mentally competent, a legally authorized representative may sign for them). A staff person must sign the witness line. It is permissible to witness the signature even if it was signed prior to arriving in the clinic, if the participant acknowledges the signature. Signatures do not need to be notarized, and it does not matter if pencil or pen is used, although pen is much preferred. A copy or duplicate of the consent form must be given to the participant or their representative. The copy does not have to be signed. The signed and witnessed original consent form must be placed in the participant's study file.

Consent for Release of Medical Information

At the same time informed consent is obtained from, family participants must also be asked to authorize release of their medical information to the HEIRS study. There is a separate form for this purpose, which can be attached to the informed consent form or maintained separately. Participants should be told that this authorization is to allow the HEIRS study to monitor their health following their CCE. It must be noted that after April 14, 2003, a separate HIPAA authorization may also be needed, so FCs should check with their Principal Investigator. Participants have the right to refuse permission, and can still be included in the study if they do so. For signatures and witnessing, follow the same procedures as the informed consent form. Be sure to print the subject's name and include their date of birth, for identification purposes. The original of this form must be kept in the participant's file. A copy does not need to be given to the participant, but they may have a copy if they ask for one.

5.7 Family Study Processes

5.7.1 Family History Form (Form 4)

1. Probands

The Family History form will be sent to all participants eligible for the CCE along with the result of the screening tests and all of the CCE material and forms. The accompanying letter will request that the Family History form and the Food Frequency Questionnaire be completed prior to the CCE visit. The study participant will be encouraged to consult with family members in order to obtain the most complete and accurate information for the Family History Form. The telephone number of a contact person at each FC will be provided so that the study participant can obtain clarification or additional information. A few days before the scheduled CCE visit, the study participant will be contacted, reminded of their appointment time and encouraged to complete all forms before the CCE visit. Upon arriving at the CCE site, the FC's designated staff person will review with the study participant the information on the Family History form, as well as the other forms. The study participant will be queried as to the relevant diseases that may have occurred in their family and the age of onset. The family history information will be used to construct the pedigree and passed along to the health care provider conducting the CCE and the person conducting genetic counseling.

2. Proband's Family Members

A proband must be identified as eligible for the Family Study. To aid in this identification, the CoC maintains lists for the FCs of CCE participants who are C282Y/C282Y and those who are provisional iron overload cases. This report is called "Potential Proband" and is located in the "tracking" menu of the HEIRS web site Each FC will only see their site specific list of participants. This report lists participants who possibly qualify as probands based on their IS and CCE lab results, and on the combination of family members from the Medical History Form. The CoC will place a "yes" in the column called "Possible Proband".

After a proband has been identified as eligible for the family study (see Section 5.3), permission will be obtained to contact their family members.. The proband will be given a reminder to contact their family members and apprise them of the study, (Reminder to Proband). The family members can be apprised of the study by the proband through personal contact, personal letters generated by the FC and signed by the proband, (Letter from Proband) or a letter generated by the FC, (Family Letter from FC). Following contact with the family member and scheduling of the CCE visit, the Family History form and the complete CCE packet of forms will be sent with an appointment confirmation letter. The accompanying letter will request that the Family History form and the other forms be completed prior to the CCE visit. At the time of the CCE visit, the family history of disease information will be reviewed and clarified by an interviewer/genetic counselor or another trained health professional. The information will be cross-checked against the information provided by the proband and the pedigree previously drawn. If the family member provides information on themselves that differs from that provided by the proband, the pedigree will be revised accordingly. The family history of disease and pedigree information will be forwarded to the health care provider conducting the CCE and the person conducting the genetic counseling.

The family members will be tracked through the "Potential Family Members" report on the HEIRS web site, in the "tracking" menu. The family members in this report will not show up until the Cyrillic data is transmitted and imported. FCs track the date the family member was called, their response, date scheduled for a CCE and date the CCE was performed.

5.7.2 Pedigree Screening Interview

Upon arriving at the CCE site, the Field Center's designated staff person will interview the study participant to review the information on the Family History form. During the interview, the form will be checked for both completeness and accuracy. Any corrections or additions will be written on the Family History form. Based on the Family History Form information, a pedigree will be drawn on the Pedigree Form (Form 8) and then reviewed with the participant. Any further corrections or additions will be made to both the Family History Form and Pedigree Form.

During the CCE interview, the Field Center's designated staff person will also assess the availability of age-eligible relatives; this information will be recorded on the Pedigree Form. If the pedigree meets eligibility criteria, then the Field Center's designated staff person will obtain permission from the potential proband to contact the available age-eligible relatives (Note: the method of contact and recruitment will vary somewhat between the Field Centers and on the preference of the proband See Table 1). **The proband and proband's relatives will be invited to participate in the Family Study only after eligibility is confirmed by the CoC and permission to contact the family members has been obtained by the proband.** Eligibility information on family members will be recorded on the Family Eligibility Form. Contact information on family members will be obtained from the potential proband and recorded on Family Contact forms. HEIRS Study participant labels will be placed on the Family History, Pedigree,

and Family Eligibility forms. Either during the CCE or within 1-2 weeks afterward, the Field Center's designated staff person will determine whether the potential proband meets all Family Study eligibility criteria by consulting the Pedigree Form, the screening test results, and the CCE results if necessary

All Family History Forms will be data-entered using the HEIRS data entry system. For probands who have agreed to participate in the Family Study, the Pedigree Forms will be data entered at the Field Centers using the Cyrillic software and then submitted electronically to the CoC via the HEIRS Study website. This can be done before or after confirmation of eligibility by the CoC. Only pedigrees obtained from Family Study probands will be data-entered.

Interviews of probands' family members will be conducted as for probands, except interviewers will have access to the proband's Family History Form and Pedigree Form information during the family member interviews. **This information from the proband will not be communicated to family members.** However, the relatives' family history and pedigree information will be cross-checked, and, if a family member provides information on themselves that differs from that provided by the proband, then the proband's pedigree will be revised accordingly. These revisions will be data-entered into the proband's pedigree in Cyrillic, and then the pedigree will be resubmitted to the CoC. The pedigree obtained from the family member will not be data-entered.

The family history information will be passed along to the health care provider conducting the CCE and the person conducting genetic counseling.

Table 1. Methods of contacting family members and completing Family Contact Form, by FC

FC	Contacting Family Members		Completing Family Contact Form	
	Using Letter from FC (Y/N)	Using Letter from Proband (Y/N)	Proband and Family Member (Y/N)*	Family Member Only (Y/N)*
UAB	Y	Y	Y	N
UCI	Y	Y	Y	N
Howard	Y	Y	Y	N
Kaiser	Y	Y	Y	N
LHSC	Y**	Y	Y	N

* FC staff may complete the Family Contact Form with assistance of designee, either in person or by phone.
** LHSC confirms that probands have gained permission from family members to be contacted.

5.7.3 Pedigree Construction

A three-generation pedigree will be constructed using standard human pedigree nomenclature (Pedigree Standardization Task Force, National Society of Genetic Counselors). The construction of an accurate family pedigree is a fundamental component of a clinical genetic evaluation and of human genetic research. Previous surveys of genetic counselors and human genetic publications have demonstrated significant inconsistencies in the usage of pedigree symbols for both common and less common scenarios. The Pedigree Standardization Task Force (PSTF) was organized to establish recommendations for universal standards in human pedigree nomenclature.

Nomenclature was chosen based on current usage, consistency among symbols, computer compatibility, and the adaptability of symbols to reflect the technical in human genetics. Usage of standardized pedigree nomenclature will reduce the chances for incorrect interpretation of patient and family medical and genetic information. It may also improve the quality of patient care provided by genetic professionals and facilitate communication between researchers involved with genetic family studies. (Bennett RL et al, 1995; Am J Hum Genet 56:745-52)

5.7.4 Pedigree Approval Process

Either during the CCE (for C282Y homozygotes) or within 1-2 weeks afterward, the Field Center's designated staff person will determine whether the potential proband meets all Family Study eligibility criteria by consulting the Pedigree Form, the genotyping test results, and the "Potential Proband" report in the tracking menu of the

web site (see Section 5.8.3.1 for explicit details). In the case of C282Y homozygous probands, family study eligibility will be determined at the CCE visit. See Pedigree Screening Interview section and Proband's Family Members section for further details.

Completed Pedigree Forms for family study probands who have agreed to participate in the Family Study will be data-entered at the Field Centers using the Cyrillic software and then submitted electronically to the CoC via the HEIRS Study web site. A hardcopy duplicate of the proband's Pedigree Form will be mailed or faxed (336-716-6427) to Leora Henkin at the CoC, along with an email (lhenkin@wfubmc.edu) to alert her that the pedigrees are being sent and how many are being sent. The faxed pedigrees need to note all family members who are likely available, as reported by the proband. This can be done by placing an "A" beside the individual ids on the pedigree. The CoC will review the pedigrees, then notify the FCs (via email) if the pedigrees are approved or not for the family study, and will also then set the tracking system reports to note that the CoC has approved the family. If any components of the pedigree have changed, then the revised pedigree should be transmitted in Cyrillic and the hard copy faxed or mailed to the CoC. The latest hard copy pedigree will be used by the CoC for data quality control checks. Also, the Family Study Subcommittee will review the structures of enrolled families and will consider changes to the family study eligibility criteria if warranted

5.7.5 Proband Eligibility Process

Probands for the Family Study will be selected from those individuals who are 25 years of age and are C282Y/C282Y or meet the study criteria for provisional iron overload after the CCE results are known.. At the CCE visit, potential probands will be evaluated as to whether they and their family structure meet the criteria for inclusion into the Family Study. Since the genotype will be known on all subjects who come in for a CCE visit those, who are C282Y/C282Y and have the appropriate family structure can be recruited at that time. If a subject has a family structure that meets the Family Study criteria, these subjects should be asked if they would be willing to participate in the Study providing all criteria are fulfilled. For these individuals, final recruitment and consent to participate in the Family Study will be conducted after the CCE indicates that they met study criteria for provisional iron overload, preferably at the time they are informed of their CCE results.

For Field Centers, the first step is to go to the web site “Tracking” menu and then to the family study report called “Potential Proband” report. Look at the Potential Proband report, as it lists participants who possibly qualify as probands, based on their IS and CCE lab data and the combination of family members on the Family History Form. Before the CoC puts a "yes" in the Possible Proband column on this list, CCE lab results must be available from the Central Lab (if the participant is not a C282Y homozygote), and CCE data must be entered by the FC, including the Family Medical History Form. FCs look at this list and decide which families to pursue based on the column that has “Yes” under the “Possible Proband”. FCs look at these families and approve their eligibility by considering the family study eligibility criteria, including the

combination of family members and the proband reports on likely available. If the FC thinks the family is eligible, then the FCs fills in the Potential Proband report column that says “FC approved Proband”.

5.7.6 Contacting Relatives of the Proband

5.7.6.1 Overview

In order to contact the relatives of the proband, permission must have been obtained to do so from the proband. The method of contact and recruitment will vary somewhat between the Field Centers and on the preference of the proband. The approach utilized should be that most acceptable to the proband and will result in the recruitment of the maximum number of eligible family members. Each Field Center will determine, based on their local protocol and the preference of the proband, which combination of the following to utilize.

5.7.6.2 Reminder Letter to Proband

The Reminder to Proband to contact their family members will be given to those probands who are either C282Y/C282Y and who also meet the family study criteria, before they leave the CCE visit. For those who are determined to be a provisional iron overload case after the CCE results are known, and who also meet family study eligibility, the Reminder to Proband to contact family members can be mailed to the proband. The probands will be asked to contact each family member, who is 19 years of age and older and for whom permission to contact has been given, and alert them that they may be contacted by the FC staff and asked to participate in the family study. Some Field Centers may request that the proband ask their relatives to contact a staff member if they are interested in participating in the Family Study. After a reasonable amount of time (typically 1-2 weeks) the proband should be contacted and asked if they have contacted their family members and if they have not, reminded to do so.

5.7.6.3 Letter from Proband

If the FC or proband prefers the Letter from Proband, it can be generated either before the proband leaves the CCE or at a later time when the proband has been found eligible for participation in the family study. Ideally the Letter from Proband will be signed by the proband and sent to the proband's family member by the FC. After sufficient time has passed to allow the proband's family members to receive information concerning the study from the proband and/or FC, the family member will be called by a staff person from the FC and asked to participate in the Family Study.

5.7.6.4 Family Letter from Field Center

The proband may be asked if the FC can contact their family members by use of the Family Letter from FC. After sufficient time has passed to allow the proband to contact their family members, the family member will be called by a staff person from the FC and asked to participate in the Family Study. Prior to this call, the proband should be called and asked if they have contacted their family members and informed them that the FC will be calling.

5.7.6.5 Phone Script for Family

The Phone Script for Family will be used as a template with each FC modifying according to local needs. After sufficient time has passed to allow the proband's family members to receive information concerning the study from the proband and/or FC, the family member will be called by a staff person from the FC and asked to participate in the Family Study.

5.7.7 Relatives' Comprehensive Clinical Examination

Approximately 2000 family members of probands who take part in the Family Study will undergo the CCE. The same procedures should be followed for the family CCE as for the regular case CCE (see Chapter 4 for CCE guidelines).

5.7.7.1 Family Study Remote Site CCE Procedures

In some circumstances, the HEIRS Family Study participants may be unwilling or unable to travel to an HEIRS Field Center for the CCE visit. In these situations, it will be necessary for the HEIRS Field Center to collaborate with local laboratories and/or health care providers for the collection, processing, and shipment of blood, and for the collection of CCE data. The following is an overview of the remote site CCE procedures.

A. Family Member Consent

1. Follow HEIRS procedures for contacting family members for participation in the Family Study.
2. Obtain verbal consent of remote family members for the Family Study.
3. Mail the Family Study Consent Form and other CCE forms (Medical History, Family History, Food Frequency Questionnaire) with stamped envelope addressed to Field Center.
4. Participant must return signed consent form before further proceeding with the Family Study CCE forms processing, blood collection, and clinical examination.

B. CCE Forms Processing, Blood Collection, and Clinical Examination

1. Participant should return the Medical History Form, Family History Form, and Food Frequency Questionnaire along with the Family Study consent form.
2. Identify local laboratory and/or health care provider to conduct the blood collection and, if possible, the clinical examination. Arrange the visit(s).
3. Review forms by phone with participant for completeness and accuracy. Obtain and record information by phone for Medical Reception section of Clinical Assessment Form.
4. Conduct Family Study pre test genetic education session by phone.
5. Follow remote site blood collection instructions and mail remote site blood collection kit to participant, the lab, or health care provider as arranged. The remote site collection kit needs to be at the collecting site **at least 24 hours** before the blood is to be collected in order for the gel pack to be frozen before transport to the Central Laboratory.
6. If a clinical examination is to be conducted, then mail examination forms (Letter to health care provider, Instructions for examination, Clinical Assessment Form, and stamped envelope addressed to Field Center) to participant..
7. If applicable, the participant must take the kit and the clinical examination packet to the lab or health care provider. (Note: It is preferable to send the lab kit directly to the remote site).
8. The local laboratory or health care provider is to send the blood samples to the HEIRS Central Laboratory, using the remote site blood collection instructions.
9. If a clinical examination is conducted, then the health care provider is to send the Clinical Assessment Form with completed Physical Exam section to the Field Center.
10. Follow HEIRS procedures for reporting CCE results to Family Study participants.
11. Conduct Family Study post test counseling by phone.

5.7.7.2 HEIRS FAMILY STUDY REMOTE SITE BLOOD COLLECTIONS

In some circumstances, the HEIRS Family Study participants may be unwilling or unable to travel to an HEIRS Field Center for the CCE visit. In these situations, it will be necessary for the HEIRS center to collaborate with local laboratories and/or health care providers for the collection, processing and shipment of blood. The HEIRS Central Laboratory will supply a remote collections kit to the Field Centers that will contain processing instructions and shipping supplies and instructions. The Field Center will add collection tubes to the kit prior to sending it to the remote site.

HEIRS Field Center Responsibilities

1. Arrangements for the CCE visit will be made by the HEIRS Field Center.

2. It is preferable to send a remote site collection and transport box directly to a local provider or a local lab so that the instructions can be reviewed prior to collecting

blood from the participant and to allow time for the gel pack to freeze (approximately 24 hours in the freezer) so that adequate temperature can be maintained during shipping. Complete the following tasks prior to sending the remote kit:

a.) Assign a bar coded HEIRS ID label and Lab ID label to the "Lab Request Form". Highlight the "Gender", "Date Specimen Collected", "Time of Specimen Collection" and "Hours Since Last Food" boxes on the form. Check the "Family Study Battery" box. Staple Lab ID labels remaining after labeling collection tubes to the form. Include this form in the kit.

b.) Include two 15 mL black and blue top Vacutainers (CPT™) labeled with the HEIRS Lab ID#. These can be placed in the 5-slotted Styrofoam container and replaced in the cardboard sleeve.

c.) Include two 10 mL lavender top Vacutainers (EDTA) labeled DNA with the HEIRS Lab ID#. These can be placed in the Styrofoam box containing the gel pack.

d.) Include two 10 mL red and gray top **double gel, plastic transport** Vacutainers (labeled with the HEIRS Lab ID#. These can be placed in the Styrofoam box containing the gel pack.

e.) Be certain that the "Local Provider and/or Laboratory Responsibilities" and "Specimen Packing and Shipping" instructions are included.

f.) Include a copy of the signed Family Study consent/medical release form from the participant.

g.) Include a pre-addressed airbill for the remote shipment to the Central Lab.

3. Fax a completed "Remote Site Shipping Log" form to the Central Laboratory once a remote collection kit is sent to a local provider or lab

Local Provider and/or Laboratory Responsibilities (Remote Site)

The detailed instructions outlined are to be followed by the local provider and/or laboratory when providing the HEIRS Field Center with specimen acquisition, processing and shipping of samples from HEIRS Family Study participants.

NOTE: Upon receipt of this mailer, remove the refrigerated Styrofoam container from its sleeving, press the gel pack into the bottom of the Styrofoam box and place in freezer for approximately 24 hours prior to specimen packing. (Leave the two larger gel packs at room temperature.)

Blood Collection and Processing

1. On the Lab Request form, complete the gender, date and time of specimen collection, and hours since last food boxes.

2. Collect six (6) tubes provided in the following order: two (2) red/gray topped tubes, two (2) lavender topped tubes, two (2) blue/black topped tubes. *Note: If there is poor venous access, fill just one red and gray topped tube followed by one lavender topped tube and then the blue and black topped tube.* Remove the tourniquet after the first tube fills.

3. Tubes #1 and #2: Two (2) 10 mL red and gray topped double gel transport tube to be filled to capacity. After drawing, allow blood to clot at room temperature for a minimum of 30 minutes. As soon as possible after 30 minutes but not longer than 45 minutes, spin tube at 1600 rcf for 10 minutes at room temperature. Place centrifuged tube in refrigerator until shipment on the same day.

4. Tubes #3 and #4: Two (2) sterile 10 mL lavender top tube (EDTA) to be filled to capacity. Invert tubes eight (8) times and store at refrigerated temperature until shipment on the same day.

5. Tubes #5 and #6: Two (2) sterile 15 mL (8 mL draw) black and blue top tube (CPT™) to be filled to capacity. Invert tubes eight (8) times and store at room temperature until shipment on the same day. These tubes should not be refrigerated.

Specimen Packing and Shipping

The remote site collection samples are shipped to the HEIRS Central Laboratory in the dual temperature combined specimen shipping boxes provided. Samples may be collected any day of the week, but Saturday and Sunday collections should be discouraged. Specimens must be shipped on the day of collection. Therefore, using the Federal Express air bill provided, mark *Priority Overnight* for shipments Saturday through Thursday and *Saturday Delivery* for shipments sent on Friday.

If packaged according to instructions supplied, this package will meet IATA guidelines for transport of diagnostic laboratory samples.

1. Place two black and blue topped tubes into the 5-slotted Styrofoam container that also contains an absorbent strip. Replace the container in the cardboard sleeve and carefully insert this box into the zip lock bag. Place this into one half of the transport box including two room temperature gel packs on either side.

2. Wrap the two lavender topped tubes and two centrifuged red/gray tubes with the sheets of paper toweling provided for protection from breakage. Place

wrapped specimens into zip lock bag, including the absorbency strip, and then into the Styrofoam container with the frozen gel pack. Put container into cardboard sleeve and place into other half of transport box.

3. Include completed HEIRS Lab Request form in the transport box. Seal the box with strapping tape. .

4. Affix the ”Diagnostic Specimens packed in compliance with IATA packaging instructions 650" label to box. Use the air bill provided and contact FedEx for pickup. (1-800-GO-FEDEX)

HEIRS Central Laboratory Responsibilities

1. The HEIRS Central Laboratory will provide a remote site transport kit with blood collection, processing and shipping instructions to the Field Centers. These kits, if packaged according to instructions supplied will meet IATA guidelines for transport of diagnostic laboratory samples.

   NOTE: Additional supplies, i.e. labeled collection tubes and laboratory request form, will be added to the kit by the HEIRS Field Center prior to sending to the remote site.

2. Upon receipt of the remote site collected samples, the Central Laboratory will fax a completed “HEIRS Remote Site Shipping Log” and the Lab Request form to the HEIRS Field Center.

3. Samples received from the Remote Site Collections will be processed by the Central Laboratory according the procedures described in Clinical Comprehensive Exam and Family Study Visit Manual of Operations: Central Laboratory Specimen Collection and Processing, August 2002 Version 1.051.

5.7.7.3 HEIRS Family Study Remote Site Shipping Log

HEIRS Central Laboratory
Phone: 612-273-3645 FAX: 612-273-3489

Please fax this completed form to the Central Laboratory whenever a remote kit is sent out. The Central Laboratory will return the same form, along with the Lab request form, by fax to the originating Field Center with the receipt date of the remote shipment.

Date Form Initiated: _____
Field Center: _____
Phone Number: _____
FAX Number: _____

Date remote kit sent to remote site:_____
Expected Date of Remote Shipment: _____
Location of Remote Site: _____
Contact Name and Number of Remote Site:
_____

Label Accession Number Assigned (HEIRS Lab ID): _____

Fed Ex Air Bill Number: _____

Central Lab Use Only:
Date of Receipt of Remote Shipment: _____

Samples received in shipment, *please circle:* 2 CPT(Blue) 2 EDTA(Lavender) 2 SST (Red/gray)

:

[ ] Form and Lab request slip faxed to _____ at (time/date)
_____

[ ] Unable to contact by fax, notice of receipt phoned at (time/date)
_____

Tech _____

5.7.8 Checks on Family Member HFE Genotypes before Reporting Results

These procedures should be followed in reporting genotyping results to family members

If a genotyping result for a family member is consistent with the family's pedigree data and all other family members' genotyping results reported to the CoC by the Central Lab, then the CoC should report this to the FC before the result is sent to the family member.

If genotyping results (including any previously reported genotyping results) are inconsistent with the family's pedigree data, then the CoC should contact the FC to identify and correct any data entry or other errors that might have caused the inconsistency. Generally, this should not involve contacting the family.

If the inconsistency is still unresolved, then the CoC and Central Lab should decide which original samples from family members should be re-genotyped. This should similarly not involve contacting the family.

If the inconsistency is unresolved after re-testing the original samples and if the inconsistency cannot be resolved by a single change in genotype within the family to C282Y/C282Y, then the FC may still report the results. The FC may also request a recommendation from the Family Subcommittee to allow collection of blood samples from one or more family members to repeat the genotyping.

Alternately, if the inconsistency is unresolved after re-testing the original samples, but the inconsistency CAN be resolved by a single change in genotype to C282Y/C282Y (i.e., a family member is potentially at high risk of iron overload, but this is not reflected in the genotyping results), then the family member should be contacted by the FC for another two-tube blood sample, for TS, SF and HFE testing, as in the initial screening.

If the inconsistency is unresolved by the second sample, then the FC may still report the results.

If the inconsistency is resolved at any stage, then the FC may report the results.

Should a participant inquire about paternity, they should be told that the test performed cannot determine paternity and there are several explanations as to why a "non-standard" test pattern was obtained.

All situations should be discussed with FC PIs in advance of any contact with participants or recollection of samples.

5.8 Data Management

5.8.1 HEIRS Family History ID Numbers

HEIRS ID numbers for family members will be assigned at the clinical sites. The first nine digits will be identical to the first nine digits in the proband's ID. The last two digits will be the family member's position in the proband's pedigree. Individuals on the pedigree are numbered from left to right starting with the first generation and working down through consecutive generations. If an individual is added to the pedigree at a later time point, then rather than renumbering individuals in the pedigree, new numbers are inserted for the new individuals. Family member ID numbers should not be reassigned without first consulting the CoC. After individuals have been numbered on the Pedigree form, these ID numbers are entered into the "office use only" section of the Family History form. If family members are added to the study, then this pedigree and family history information should be updated accordingly.

5.8.2 Pedigree Data Entry

During the CCE, clinics will collect Family History Forms from each participant. These forms will then be reviewed and clarified by a genetic specialist or another health trained professional. A pedigree will be constructed on the Pedigree Form from the information provided, and a determination will be made regarding the appropriateness of the family structure for inclusion in the family study. The participant will also be queried as to the potential availability and willingness of specific age-eligible family members to participate in the family study. This should be indicated by placing an "A" beside the individual id on the pedigree. This information will be recorded on the Family Eligibility Form. Pedigree Forms will be data-entered at the Field Centers using the Cyrillic database provided by the CoC. The two-digit individual number (described in section 5.8.1) will be the individual ID entered into the Cyrillic database. Following data entry or update, the HEIRS Family Study database will be submitted to the CoC via the HEIRS Study web site.

5.8.3 Tracking Family Recruitment and Pedigrees:

The CoC will track recruitment for the family study for each proband and family member in the HEIRS Tracking System (Under the "Tracking" menu). Only participants who are C282Y homozygotes or those who have met the study definition of provisional case are eligible to be probands in the family study. CoC confirmation of proband and family eligibility will be entered by the CoC in the Tracking System. Please see the section below (5.8.3.1) for explicit tracking procedures for the family study.

Focus for recruitment will be on CCE visits by family members in the optimal family structure (offspring, non-proband parent of any offspring, full siblings, parents).

In addition, the CoC will provide drawings of each approved pedigree to the Family Study Subcommittee for review. The Family Study Subcommittee will consider changes to the optimal family structure criteria if warranted.

5.8.3.1 Family Study Tracking Procedures

1. The first step is to go to the web site “Tracking” menu and then to the family study reports section: “Potential Proband” report and “Potential Family Members” report.
2. Look at the Potential Proband report, as it lists participants who possibly qualify as probands, based on their IS and CCE lab data and the combination of family members on the Family History Form. Before the CoC puts a "yes" in the Possible Proband column on this list, CCE lab results must be available from the Central Lab (if the participant is not a C282Y homozygote), and CCE data must be entered by the FC, including the Family Medical History Form.
3. FCs look at this list and decide which families to pursue based on the column that has “Yes” under the “Possible Proband”. FCs look at these families and approve their eligibility by considering the family study eligibility criteria, including the combination of family members and the proband reports on likely available. If the FC thinks the family is eligible, then the FCs fills in the Potential Proband report column that says “FC approved Proband”.
4. The applicable pedigrees are then faxed to Leora Henkin at the CoC for approval at 336-716-6427. The faxed pedigrees need to note all family members who are likely available, as reported by the proband. This can be done by placing an "A" beside the individual ids on the pedigree. An email is then sent to Leora (lhenkin@wfubmc.edu) stating how many pedigrees are being sent. FCs also data enter the date the pedigrees were faxed to the CoC in the “Potential Proband” report column that says, “Pedigree faxed to CoC”.
5. The CoC will review the pedigrees, then notify the FCs (via email) if the pedigrees are approved or not for the family study, and will also then set the tracking system reports to note that the CoC has approved the family. Only the CoC can indicate “CoC approved”.
6. FCs then data enter the pedigrees into Cyrillic and submit to the CoC. There is new web site report available that will assist FCs in keeping track of when they transmitted Cyrillic and when the CoC approved the pedigrees. These are FC specific reports and are located under the “Reports” menu and the “Family Study” section and are called “Pedigree Approval Tracking – List of Probands.” Each FC has their own site specific report. The date that the Cyrillic was sent is listed here, as well as the date the pedigree was CoC approved, the proband ethnicity and the HFE status.
7. Pedigree information is imported by the CoC into the tracking system in the Potential Family Members report. The family members will not appear in this report until the Cyrillic data is transmitted and imported.
8. FC should follow all FC-specific procedures for contacting family members. However, it is at this point that FCs typically may begin contacting the family members and inviting them to have a CCE. FCs should track this using the Potential Family Members report, and enter the date they were called, their response, date they were scheduled for a CCE, date they attended the CCE, etc. Each family member should be tracked through this report. This is the same procedure for the case and control CCE tracking.

9. FCs should update hardcopy pedigrees and Cyrillic data on families participating in the family study. Hardcopy updates should be faxed to Leora for CoC files. Cyrillic updates should be sent to the CoC via HEIRS the web site.

6. GENETIC COUNSELING

6.1 Overview

"Genetic counseling is a communication process which deals with the human problems associated with the occurrence or risk of occurrence of a genetic disorder in a family. This process involves an attempt by one or more appropriately trained persons to help the individual or family to: 1) comprehend the medical facts including the diagnosis, probable course of the disorder, and the available management, 2) appreciate the way heredity contributes to the disorder and the risk of recurrence in specified relatives, 3) understand the alternatives for dealing with the risk of recurrence, 4) choose a course of action which seems to them appropriate in their view of their risk, their family goals, and their ethical and religious standards and act in accordance with that decision, and 5) to make the best possible adjustment to the disorder in an affected family member and/or to the risk of recurrence of that disorder." (This is the definition of genetic counseling adopted by the American Society of Human Genetics in 1975 and which still stands today.)

Genetic assessment, education, information, and/or counseling will be provided to thousands of individuals who will be screened as a part of the HEIRS project. This chapter is designed to clarify what genetic information or counseling will be provided and to which individuals. The purposes of this chapter are:

1) to stipulate the points in the study when genetic counseling is necessary, 2) to delineate the method(s) by which genetic counseling should take place, and 3) to specify the information that should be imparted.

While some people require services and counseling from a specialist trained in genetics (e.g. a geneticist or genetic counselor), others will receive genetic information and education from other physicians, research associates, or clinic coordinators. For the purposes of the HEIRS study, each Field Center (FC) will use its own discretion to decide who performs the counseling. It will be critical that all members of the HEIRS staff be trained to respond to basic genetic questions and to recognize when they need to seek advice or further information from the genetics specialist. Further, it will be important to be able to determine when referral to a genetics specialist is indicated. Because genetic information given to participants will shape their understanding of the implications of their genetic test results for themselves and other family members and hence their responses to the screening experience and the ELSI assessments, it is important that all centers provide the same information and adhere to the same genetic counseling protocols.

Genetic counseling is performed at the CCE for subjects who screened positive for genotype (C282Y homozygotes) or phenotype (potential iron overload), and for members of the family study. The guidelines for genetic counseling at the CCE, including content, are contained in the Genetic Counseling Checklist, which was revised in May 2002. The checklist (provided in Section 6.5) should be used to assist in ensuring complete coverage of these necessary topics.

6.2 Guidelines and Procedures Regarding Counseling

6.2.1 Background Information for Recruiters and Subjects

As outlined in the Genetics Education and Counseling chapter of the HEIRS Protocol, education materials developed jointly with the Recruitment and Retention Committee will be available at each site. The genetic counselor(s) or geneticist(s) at each site will train recruiters to provide basic information regarding the genetics of Hemochromatosis and significance of various possible test results. This will enable recruiters to answer questions that may arise during the process of informed consent.

6.2.2 Screening Results Notification/ Invitation to Participate in CCE

All participants will be notified of their TS, SF, and HFE initial screening (IS) results. The templates for results letters should be used when letters are sent, and specific laboratory values must be given. The manner of notification may differ by FC, but each center will contact subjects qualifying for the CCE by phone, and send a follow-up letter confirming an appointment time and including instructions for the clinic visit as well as other forms. Individuals who do not qualify for the CCE, will be sent a letter explaining that they will not need to return for a CCE on the basis of unusual results, but may be asked to participate in the study as a control. They should be thanked for participating in the study. Participants coming for CCEs as controls will not receive genetic counseling. The name and phone number of the study coordinator at the site should be included in all written communications.

It is especially important to provide educational materials for individuals who do not qualify for the CCE but are genotype positive, i.e., heterozygotes, C282Y/H63D compound heterozygotes and H63D homozygotes. These materials should include the information about the relevant genotype, its implications (if any) for first degree relatives and future generations, and the clinical features, natural history, and management options for HH/IO.

6.2.3 Family History and Pedigree at CCE

CCE subjects (but not controls) will receive a family history form with their appointment confirmation letter. They should be asked to complete this form and bring it to their CCE visit (see Chapter 4 for complete CCE procedures). At the CCE, the family history form will be reviewed by the genetic counselor, nurse, or other trained individual prior to the examination, and necessary additions/clarifications should be made. While the subject is being examined, the genetic counselor, nurse, or other trained personnel will construct a three generation pedigree using the family information form. Analysis of this pedigree will help to determine eligibility for participation in the family study (see Family Study Eligibility Criteria, Chapter 5). If the family structure meets these criteria, the subject should be asked about the willingness and potential availability of other family members to participate in the family study.

6.3. HEIRS Genetic Counseling Content

6.3.I. Introduction

Genetic counseling is provided at the CCE to participants who screened positive for genotype (C282Y homozygote) or phenotype (potential iron overload), and for members of the family study. The checklist provided in Section 6.5 should be used to assist in ensuring complete coverage of these topics.

6.3.2. Content of Genetic Counseling Sessions at the CCE

The elements to be covered in the genetic counseling session for CCE cases fall into the following categories:

- review of family history and pedigree construction,
- discussion of basic information about the features and genetics of Hemochromatosis,
- discussion of the participant's genotype and its implications for the participant's own health and that of other family members,
- discussion of genetic discrimination issues and legal protections or exclusions—including those regarding blood donation,
- exploration of the psychosocial impact of test results and clinical findings.

The information provided to participants with various genotypes will have to be modified according to a number of factors including: (a) whether there was a previous known diagnosis of Hemochromatosis in the participant or another family member; (b) the presence or absence of iron overload in the participant and additional findings from the participant's CCE such as the presence or absence of symptoms potentially related to iron overload; (c) other possible genetic causes of secondary iron overload (e.g., thalassemia);and (d) and the participant's age and gender.

For **'genotype positive' participants** (those who carry at least one HFE mutation), information presented should include: health problems that can be associated with the subject's genotype and the likelihood of these occurring [all but some C282Y/C282Y homozygotes coming for CCE will already have had screening for possible iron overload] and genotypes that may be found in other family members and the likelihood of each occurring.

- health problems (if any) that could potentially be associated with these other possible genotypes. The following information regarding health problems potentially associated with specific genotypes can be provided as appropriate:

  (1) <u>C282Y/C282Y</u> (C282Y homozygote) – A person with this genotype carries two copies of the major HFE mutation. C282Y homozygotes represent at least 75% (in some populations >90%) of people with hemochromatosis. Expression of the disease ranges from no evidence of iron overload in some to massive iron overload with organ dysfunction in others. About half of

people with this genotype will go on to develop symptoms related to iron overload [Burke, et al]. Health problems occur due to accumulation of iron in the liver, heart, pancreas, joints or pituitary gland and can include fatigue; heart problems; diabetes; arthritis; change in skin color to bronze, tan, or grey; sexual problems such as impotence in men or infertility and amenorrhea in women; cirrhosis of the liver or liver cancer. In an asymptomatic person, early treatment usually prevents symptoms from developing. In a symptomatic person, treatment usually improves energy level, stabilizes liver disease and may reduce heart disease and thus ease breathing. However,

(2) treatment cannot usually reverse diabetes or arthritis and may not cure impotence (although this may improve with testosterone or Viagra).

(3) C282Y/H63D (compound heterozygote) – People with this genotype have one copy of the major HFE mutation and one copy of the minor, more common and less harmful mutation. Most individuals with this genotype have normal iron studies. A small proportion have mild to moderate iron overload. Severe iron overload usually occurs only when there is another risk factor such as hepatitis or alcoholism.

(4) H63D/H63D (H63D homozygote) – A person with this genotype carries two copies of the minor HFE mutation. Most people with this genotype have normal iron studies, although a few have mild to moderate iron overload. Severe iron overload usually occurs in H63D homozygotes only when there is another risk factor such as hepatitis or alcoholism.

(5) C282Y heterozygote (C282Y carrier) – Most C282Y carriers have one copy of the major HFE mutation and a copy of the normal HFE gene. A few may carry a second undetectable HFE mutation instead of the normal HFE gene. About 8% of the Caucasian population are C282Y heterozygotes. C282Y carriers usually have normal iron levels. In rare cases iron overload can occur, probably because the individual also carries a second undescribed or undetected HFE mutation.

(6) H63D heterozygote (H63D carrier) – A person with this genotype carries one copy of the minor HFE mutation and and one copy of the normal HFE gene. A few may carry a second undetectable HFE mutation instead of the normal HFE gene. This genotype is seen in about 23% of the population. If an H63D carrier has iron overload, it is probably due to something else.

(7) normal HFE homozygote, with iron overload – A person with this genotype has none of the HFE mutations that were tested for. Other harmful mutations in HFE may be discovered in the future, as may different genes predisposing to iron overload. If a person with no detectable HFE mutations has iron overload, he/she should be evaluated to look for other causes and determine if treatment is necessary. In certain non-European populations (Africans, African-Americans, Chinese, Polynesians), iron overload can run in families—presumably because of mutations in one or more genes besides HFE.

The above information and the likelihood of encountering various genotypes in siblings, parents and offspring is summarized in the following tables for Caucasian probands who are C282Y/C282Y, C282Y/H63D, and H63D/H63D, C282Y/+, H63D/+ and +/+ respectively.

TABLE I
CCE and ELSI Cases, ELSI-only Cases, and CCE and ELSI Controls
(please note that a (+) in this table indicates no HFE mutation detected)

IRON LEVELS MEETING CCE THRESHHOLD	IRON LEVELS BELOW CCE THRESHHOLD
GROUP #1: C282Y/C282Y Potential CCE and ELSI	GROUP #7: C282Y/C282Y Potential CCE and ELSI cases
GROUP #2: C282Y/H63D Potential CCE and ELSI cases	
GROUP #3: H63D/H63D Potential CCE and ELSI cases	
GROUP #4: C282Y/+ Potential CCE and ELSI cases	
GROUP #5: H63D/+ Potential CCE and ELSI cases	
GROUP #6: +/+ Potential CCE and ELSI cases	GROUP #12: +/+ Potential CCE and ELSI Controls

TABLE II
IF THE PROBAND YOU ARE COUNSELING IS C282Y/C282Y (Groups 1 and 7)

This genotype is found in at least 75% of people with hemochromatosis. Iron overload and/or symptoms are likely to be related to genotype. The person you are counseling may currently be either symptomatic or asymptomatic, but is at significant risk for iron overload.

	C282Y/C282Y	H63D/H63D	C282Y/H63D	C282Y/+	H63D/+	+/+
HEALTH IMPLICATIONS	Health risk ranges from no evidence of iron overload to massive iron overload with organ damage. About half of individuals with this genotype will go on to develop symptoms related to iron overload.	Most individuals have normal iron levels. A small proportion have mild to moderate iron overload, usually in the presence of another risk factor, e.g. hepatitis or alcoholism.	Most individuals have normal iron levels. A small proportion have mild to moderate iron overload, usually in the presence of another risk factor, e.g. hepatitis or alcoholism.	Individuals usually have normal iron levels. In rare cases there is iron overload, possibly because of an undescribed, second mutation.	Individuals should have normal iron levels. If there is iron overload, it is probably due to another cause.	
RISKS TO SIBLINGS	About 1 in 4 (26%)	About 1 in 170 (0.6%)	About 1 in 13 (8%)	About 1 in 2 (42%)	About 1 in 16 (6%)	About 1 in 6 (17%)
RISKS TO ONE OR BOTH PARENTS	About 1 in 18 (6%)	No chance, unless non-paternity	About 2 in 7 (28%)	Nearly all (97%)	No chance, unless non-paternity	No chance, unless non-paternity
RISKS TO OFFSPRING	About 1 in 20 (5%)	No chance, unless non-paternity	About 1 in 7 (15%)	About 4 in 5 (80%)	No chance, unless non-paternity	No chance, unless non-paternity

TABLE III
IF THE PROBAND YOU ARE COUNSELING IS H63D/H63D
(Group 3)

This genotype is seen in a small proportion of people with hemochromatosis. Most people with this genotype do not have symptoms of iron overload. If iron overload and/or symptoms are present, they may either be related to the genotype or to other risk factors.

	C282Y/C282Y	H63D/H63D	C282Y/H63D	C282Y/+	H63D/+	+/+
HEALTH IMPLICATIONS	Health risk ranges from no evidence of iron overload to massive iron overload with organ damage. About half of individuals with this genotype will go on to develop symptoms related to iron overload.	Most individuals have normal iron levels. A small proportion have mild to moderate iron overload, usually in the presence of another risk factor, e.g. hepatitis or alcoholism.	Most individuals have normal iron levels. A small proportion have mild to moderate iron overload, usually in the presence of another risk factor, e.g. hepatitis or alcoholism.	Individuals usually have normal iron levels. In rare cases there is iron overload, possibly because of an undescribed, second mutation.	Individuals should have normal iron levels. If there is iron overload, it is probably due to another cause.	
RISKS TO SIBLINGS	Less than 1 in 1000 (<1%)	About 1 in 3 (30%)	About 1 in 30 (3%)	About 1 in 40 (2.6)%	About 1 in 2 (46%)	About 1 in 5 (18%)
RISKS TO ONE OR BOTH PARENTS	No chance, unless non-paternity	About 1 in 6 (16%)	About 1 in 9 (11%)	No chance, unless non-paternity	Almost all (98%)	No chance, unless non-paternity
RISKS TO OFFSPRING	No chance, unless non-paternity	About 1 in 7 (15%)	About 1 in 20 (5%)	No chance, unless non-paternity	About 4 in 5 (80%)	No chance, unless non-paternity

TABLE IV
IF THE PROBAND YOU ARE COUNSELING IS C282Y/H63D (Group 2)

This genotype is seen in a small proportion of people with hemochromatosis. Most people with this genotype do not have symptoms of iron overload. If iron overload and/or symptoms are present, they are probably related to the genotype but may be due to other risk factors.

	C282Y/C282Y	H63D/H63D	C282Y/H63D	C282Y/+	H63D/+	+/+
HEALTH IMPLICATIONS	Health risk ranges from no evidence of iron overload to massive iron overload with organ damage. About half of individuals with this genotype will go on to develop symptoms related to iron overload.	Most individuals have normal iron levels. A small proportion has mild to moderate iron overload, usually in the presence of another risk factor, e.g. hepatitis or alcoholism.	Most individuals have normal iron levels. A small proportion has mild to moderate iron overload, usually in the presence of another risk factor, e.g. hepatitis or alcoholism.	Individuals usually have normal iron levels. In rare cases there is iron overload, possibly because of an undescribed, second mutation.	Individuals should have normal iron levels. If there is iron overload, it is probably due to another cause.	
RISKS TO SIBLINGS	About 1 in 70 (1.4%)	About 1 in 25 (4%)	About 2 in 7 (28%)	About 1 in 4 (23%)	About 1 in 4 (26%)	About 1 in 5 (18%)
RISKS TO ONE OR BOTH PARENTS	About 1 in 35 (2.8%)	About 1 in 12 (8%)	About 1 in 5 (20%)	About 4 in 5 (82%)	About 9 in 10 (86%)	No chance, unless non-paternity
RISKS TO OFFSPRING	About 1 in 36 (3%)	About 1 in 13 (7.5%)	About 1 in 10 (10%)	About 2 in 5 (40%)	About 2 in 5 (40%)	No chance, unless non-paternity

Although normal homozygotes and carriers are much less likely to be cases, they may come for CCE as controls or be identified in the course of family studies. For this reason, information regarding implications for their health and relatives is summarized in the following three tables.

TABLE V
IF THE PROBAND YOU ARE COUNSELING IS A C282Y HETEROZYGOTE (Group 4)

This genotype is only rarely seen in people with hemochromatosis. If iron overload and/or symptoms are present, the may related to the genotype (which may include an undetected second mutation), or to other risk factors.

	C282Y/C282Y	H63D/H63D	C282Y/H63D	C282Y/+	H63D/+	+/+
HEALTH IMPLICATIONS	Health risk ranges from no evidence of iron overload to massive iron overload with organ damage. About half of individuals with this genotype will go on to develop symptoms related to iron overload.	Most individuals have normal iron levels. A small proportion have mild to moderate iron overload, usually in the presence of another risk factor, e.g. hepatitis or alcoholism.	Most individuals have normal iron levels. A small proportion have mild to moderate iron overload, usually in the presence of another risk factor, e.g. hepatitis or alcoholism.	Individuals usually have normal iron levels. In rare cases there is iron overload, possibly because of an undescribed, second mutation.	Individuals should have normal iron levels. If there is iron overload, it is probably due to another cause.	
RISKS TO SIBLINGS	About 1 in 70 (1.5%)	About 1 in 100 (1%)	About 1 in 16 (7%)	About 1 in 2 (44%)	About 1 in 9 (12%)	About 1 in 3 (34%)
RISKS TO ONE OR BOTH PARENTS	About 1 in 33 (3%)	No chance, unless non-paternity	About 1 in 7 (15%)	About 5 in 6 (83%)	About 1 in 4 (26%)	About 2 in 3 (69%)
RISKS TO OFFSPRING	About 1 in 36 (2.7%)	No chance, unless non-paternity	About 1 in 13 (7.5%)	About 3 in 7 (43%)	About 1 in 13 (7.5%)	About 2 in 5 (40%)

TABLE VI

IF THE PROBAND YOU ARE COUNSELING IS AN H63D HETEROZYGOTE (Group 5)

This genotype is only rarely seen in people with hemochromatosis. If iron overload and/or symptoms are present, they may rarely be related to the genotype (perhaps one that includes an undetected second mutation), or more likely be due to other risk factors.

	C282Y/C282Y	H63D/H63D	C282Y/H63D	C282Y/+	H63D/+	+/+
HEALTH IMPLICATIONS	Health risk ranges from no evidence of iron overload to massive iron overload with organ damage. About half of individuals with this genotype will go on to develop symptoms related to iron overload.	Most individuals have normal iron levels. A small proportion have mild to moderate iron overload, usually in the presence of another risk factor, e.g. hepatitis or alcoholism.	Most individuals have normal iron levels. A small proportion have mild to moderate iron overload, usually in the presence of another risk factor, e.g. hepatitis or alcoholism.	Individuals usually have normal iron levels. In rare cases there is iron overload, possibly because of an undescribed, second mutation.	Individuals should have normal iron levels. If there is iron overload, it is probably due to another cause.	
RISKS TO SIBLINGS	Negligible (<1%)	About 1 in 22 (4.5%)	About 1 in 32 (3.2%)	About 1 in 20 (5%)	About 1 in 2 (50%)	About 2 in 5 (37%)
RISKS TO ONE OR BOTH PARENTS	No chance, unless non-paternity	About 1 in 11 (9%)	About 1 in 15 (6.6%)	About 1 in 10 (10%)	About 9 in 10 (88%)	About 3 in 4 (74%)
RISKS TO OFFSPRING	No chance, unless non-paternity	About 1 in 13 (7.7%)	About 1 in 39 (2.6%)	About 1 in 36 (2.7%)	About 1 in 2 (47%)	About 2 in 5 (41%)

TABLE VII
IF THE PROBAND YOU ARE COUNSELING IS A NORMAL HOMOZYGOTE (Group 6)

No HFE mutations were detected. If iron overload and/or symptoms are present, they are either due to other risk factors, or to one or more changes in as yet unidentified other genes.

	C282Y/C282Y	H63D/H63D	C282Y/H63D	C282Y/+	H63D/+	+/+
HEALTH IMPLICATIONS	Health risk ranges from no evidence of iron overload to massive iron overload with organ damage. About half of individuals with this genotype will go on to develop symptoms related to iron overload.	Most individuals have normal iron levels. A small proportion have mild to moderate iron overload, usually in the presence of another risk factor, e.g. hepatitis or alcoholism.	Most individuals have normal iron levels. A small proportion have mild to moderate iron overload, usually in the presence of another risk factor, e.g. hepatitis or alcoholism.	Individuals usually have normal iron levels. In rare cases there is iron overload, possibly because of an undescribed, second mutation.	Individuals should have normal iron levels. If there is iron overload, it is probably due to another cause.	
RISKS TO SIBLINGS	Negligible (<1%)	About 1 in 65 (1.5%)	About 1 in 90 (1.1%)	About 1 in 13 (7.5%)	About 1 in 5 (21%)	About 7 in 10 (69%)
RISKS TO ONE OR BOTH PARENTS	No chance, unless non-paternity	No chance, unless non-paternity	No chance, unless non-paternity	About 1 in 6 (17)%	About 3 in 7 (44%)	About 9 in 10 (88%)
RISKS TO OFFSPRING	No chance, unless non-paternity	No chance, unless non-paternity	No chance, unless non-paternity	About 1 in 18 (5.5)%	About 1 in 7 (15%)	About 4 in 5 (80%)

6.4. Genetics Consultation for Family Members

After the examination, the subject will meet with the genetic counselor, clinical geneticist or other physician/nurse for counseling. Most participants will not have their genotype results available at the time of the CCE, so the counseling will usually be limited to basic information about Hemochromatosis and iron overload and the associated HFE mutations. The discussion should include:

1. possible genotype results and information about prevalence, mode of inheritance, natural history, recurrence risk, implications for other family members, and methods available for diagnostic and carrier testing,
2. review of finalized pedigree with attention to history suggestive of iron overload and related diseases, emphasizing our limited knowledge about other genetic and environmental influences and interactions in the development of IO
3. information regarding local/national support organizations
4. information regarding surveillance for, and management of IO
5. exploration of the subject's concerns and feelings

6.4.2 Guidelines for Discussing Test Results with Family Members

Although a few family members (FMs), who have already been screened, will have information about their genotype and iron levels when they come for a CCE, most participants will not have this information. Some FMs may have learned about a relative's genotype or phenotype. But since specific genetic counseling for FMs will not usually be able to be provided until after they have received results from the CCE and the lab work, their counseling at the CCE will usually be limited to providing general information about the purposes of the study and Hemochromatosis genetics. After the FMs laboratory and other clinical findings are available, FCs can determine how best to provide this counseling. At that point, the same elements should be covered as with the probands at the CCE. In either situation, genetic counseling for FMs should be based only on their results and not on information that has been learned about their family members during the study. An exception can be made if the proband or other relative has divulged their results to the FM. In this case, the following guidelines dictate what can be shared in specific circumstances:

At the CCE

In normal cases, family members will **NOT** have their test results, so counseling will not be done. Instead, FMs can be given some general background information about the purposes of the study and the genetics of hemochromatosis. This can be done simply by giving them the handouts that some FCs are using at initial screening, and answering any questions they have. FMs should not be told anything about the proband's or other FMs' results; unless

there is evidence that these results have been shared with them by that relative. If they ask, they should be told they need to raise those questions directly with their relative since patient confidentiality prevents HEIRS personnel from giving that information to anyone else.

If the FM Already Has Screening Results. Some FMs may have already come through the initial screening phase, in which case they should have received their Initial Screening results letter and their screening results should be available at the CCE. In this case, they should be given the same type of genotype-specific counseling that is usually given at the CCE. If the FMs IS results are normal, then they can simply be given the background information about IO and HH.

If the FM Has Information about the Proband's Results. See below.

After the CCE

FC's can pursue one of 3 strategies in counseling Family Members about their results when the CCE results become available:

Letter: At a minimum, a letter explaining CCE results and offering an *opportunity* for counseling, either by phone or in person, if the subject desires, should be sent. For C282Y homozygotes, the letter should *recommend* counseling by the FC. Also, for those with elevated iron levels, the letter should strongly urge the FM to seek clinical follow-up, although this need not be done at the FC.

Phone: FC's are encouraged to make affirmative efforts to contact FMs who are C282Y homozygotes or who have elevated iron levels by telephone.

In Person: FC's may, if they wish, make affirmative efforts to bring back FMs who are C282Y homozygotes or who have elevated iron levels for in-person counseling and clinical follow-up.

What Information Can and Cannot be Given to Family Members. In all of these situations, counseling and communication of results will be similar to how it is done with potential cases at the CCE. In other words, counseling should be based only on the FM's results rather than using information from the proband or other family members. The following guidance applies to special situations:

If a FM presents information about a relative's results (e.g., the Proband's results) that were given by that relative, it is permissible for clinic staff to answer questions about what that information means for the FM. However, clinic staff should not provide any

new information about the FM's relatives and the communication should still focus on the FM's own results.

If the FM has a copy of the Proband's (or another family member's) results letter, it is permissible to discuss the contents of the letter to the extent of repeating or clarifying what it says. However, the FM's possession of the letter does not mean that their relative has given unrestricted permission to discuss with the FM the significance of the letter as it relates to the Proband or other family members.

It is always permissible to provide a general discussion of the genetics of hemochromatosis, which may cover concepts that overlap with the significance of the letter as it relates to the Proband or other family members.

In **no** event should clinic staff discuss with subjects any issues relating to possible "false paternity," or other genetic evidence that suggest that family relationships are not as they were reported (for example, genotype results that show that someone's father is not who they thought he was).

6. 4.3. Post-Test Results Notification of Family Members

Family members will be notified of test results either by phone or by letter, depending on FC protocol. It is not anticipated that participating family members will return to the FC for face-to-face genetic counseling unless they are unduly distressed or they specifically request to return. The genetic counselor or designee will be available by phone for individuals who have questions/concerns. Please see previous section for guidelines.

One week after CCE test results are provided to family members, the “Family Post Result” ELSI assessment forms should be mailed to family members. Note that the ELSI assessment form for family members is different than it is for other participants. The Family Post Result Form will be included in the HEIRS web site tracking system. As with the other ELSI Post Result forms, a second mailing will be done if the participant does not send back the form with the initial mailing. The tracking system will include a column indicating when to send the second mailing, if the first mailing was not received. The CoC inserts this date, so it is important for the FCs to enter data into the tracking system in a timely manner. Please refer to Chapter 11 for explicit instructions and more details on the tracking system and data entry guidelines.

6.5 Genetic Counseling Checklist

Genetic Counseling Checklist for Subjects at the CCE

Subject's ID number: ___ ___ - ___ ___ - ___ ___ ___ ___ ___ - ___ ___
Counselor: _____ Date _____

Items in boldface should be done or reviewed with <u>all</u> CCE cases; others can be discussed at the counselor's discretion or when the situations in italics apply.

1. **review or construction of pedigree**
   - ☐ reviewed by genetics specialist
   - ☐ constructed by genetics specialist
2. ***assessment of family's eligibility for family study (indicate which situation applies)***
   - ☐ meets ***revised*** minimal criteria [1) proband C282Y/C282Y <u>or</u> meeting HEIRS Study criteria for 1° iron overload, regardless of genotype; <u>and</u> 2) available family members of ≥19 y.o. including at least a) four first degree relatives of the proband; <u>or</u> b) three offspring of the proband by the same mating; <u>or</u> c) two full siblings of the proband]
   - ☐ meets optimal criteria [nuclear family of at least four age-eligible individuals, of whom at least one is a parent <u>and</u> at least eight additional age-eligible 1° relatives of nuclear family members; all must be available for study]
   - ☐ is not eligible as proband for family study [insufficient number of family members ≥19; proband has 2° iron overload; biological relative of subject is already proband for family study]
3. **review of subject's genetic test results (check only one)**
   - ☐ C282Y/C282Y
   - ☐ H63D/H63D
   - ☐ C282Y/H63D
   - ☐ C282Y/WT
   - ☐ H63D/WT
   - ☐ WT/WT
4. **review of subject's biochemical test results**
   - ☐ serum ferritin (indicate level) _____
   - ☐ transferrin saturation (indicate level) _____
5. **information about hemochromatosis inheritance (review with <u>all</u> "genotype positive" subjects and with others as deemed appropriate)**
   - ☐ most due to autosomal recessive gene, *HFE*
   - ☐ homozygote or compound heterozygote inherits mutations from <u>both</u> parents
   - ☐ incomplete penetrance with sex and age effects
   - ☐ two common major mutations with differing expression and other less common mutations
   - ☐ population differences in incidence (about a quarter of N. European Caucasians carry at least one copy of C282Y or H63D)
   - ☐ probability that one or more different genes cause iron overload in other populations or can modify the effects of C282Y and H63D

6. **information about hemochromatosis symptoms *(when subject has high likelihood of IO)***
   - occur because body absorbs too much iron, which accumulates in organs and tissues (liver, heart, pancreas, joints, pituitary gland)
   - health problems can include fatigue, heart problems, diabetes, arthritis, change in skin color, decreased sexual drive, impotence or amenorrhea, cirrhosis of the liver or liver cancer
   - likelihood of symptoms increases with age and is greater in males
   - some people with genetic predisposition will never develop symptoms or health problems – even with biochemical evidence of iron overload
7. **information about hemochromatosis treatment *(when subject has high likelihood of IO)***
   - phlebotomy effective if iron overload is present (removal of up to one pint per week until levels return to normal)
   - initial treatment may take from a few months for a young woman to up to three years for an older man with severe iron overload
   - subsequently may need only a few phlebotomies a year
   - dietary changes to reduce risk of hepatitis or liver damage (avoid raw fish or shellfish; reduce alcohol consumption to <2 drinks per day or abstain if liver damage is present)
   - reduce iron absorption (reduce alcohol consumption; take ≤500 mg. of vitamin C per day; reduce red meat consumption)
   - drug therapy not indicated since phlebotomy usually effective
8. **discussion of implications of genetic test results for subject's health (complete only <u>one</u> of the following sections; indicate which applies by checking box in front of genotype)**

 C282Y/C282Y with or without iron overload
   - expression ranges from no evidence of iron overload to massive iron overload
   - about half of people who are C282Y/C282Y will eventually develop symptoms if iron overload is untreated
   - in an asymptomatic person, treatment usually prevents symptoms from developing
   - in a symptomatic person, treatment usually improves energy level, may reduce heart disease and stabilize liver disease, but cannot usually reverse diabetes or arthritis and may not cure impotence

 H63D/H63D or C282Y/H63D with elevated TS and ferritin
   - the elevated iron levels may or may not be due to the subject's genotype
   - when iron overload occurs in individuals who are H63D/H63D or C282Y/H63D, it is usually mild to moderate
   - severe iron overload usually occurs only with another risk factor such as hepatitis or alcoholism
   - other reasons for the increased iron levels may be identified by further tests

 C282Y/WT with elevated TS and ferritin
   - abnormal iron levels may or may not be due to being a C282Y carrier
   - other reasons for the increased iron levels may be identified by further tests

- when iron overload occurs in C282Y carriers, they may have a second HFE mutation that is not yet recognized or was not tested for by this study
- *H63D/WT with elevated TS and ferritin*
  - abnormal iron levels are probably not due to being a H63D carrier
  - other reasons for the increased iron levels may be identified by further tests
- *<u>WT/WT with elevated TS and ferritin</u> (for <u>all</u> subjects with no detectable HFE mutation)*
  - abnormal iron levels are not due to any HFE mutations that could be detected by testing done in this study
  - other reasons for the increased iron levels may be identified by further tests
  - some individuals, particularly in non-European population groups, may have mutations in a different gene or genes that increase iron absorption

9. **discussion of implications of biochemical test results for subject's health (review when iron levels are high and no clear reason is apparent)**
   - reasons for the increased iron levels may be identified by further tests and results will be communicated to them by the physician or field center coordinator
10. **discussion of possible implications of genetic test results for other family members (check appropriate genotype and complete only one of the following sections).**

Note: frequencies apply only to participants whose families are of European Caucasian descent. For non-Caucasian probands, use last checkbox under WT/WT with elevated TS and ferritin.

- **C282Y/C282Y with or without iron overload**
  - Chance for sibling of Caucasian proband to be C282Y/C282Y and thus have at least a 50% risk to develop iron overload = 26% (about 1 in 4)
  - Chance for sibling of Caucasian proband to be at slightly increased risk for iron overload due to H63D/H63D = 0.6% (about 1 in 170); or to C282Y/H63D = 8% (about 1 in 13)
  - Chance for sibling of Caucasian proband to be an HFE mutation carrier C282Y/WT = 42% (about 1 in 2); or H63D/WT = 6% (about 1 in 16)
  - Chance for sibling of Caucasian proband to be a normal homozygote = 17% (about 1 in 6)
  - Chance for one or both parents of Caucasian proband to be C282Y/C282Y and thus have at least a 50% risk to develop iron overload = 6% (about 1 in 18)
  - Chance for one or both parents of Caucasian proband to be at slightly increased risk for iron overload due to C282Y/H63D = 28% (about 2 in 7)
  - Chance for one or both parents of Caucasian proband to be an C282Y carrier = 97% (nearly all)
  - Chance for child of Caucasian proband to be C282Y/C282Y and thus have at least a 50% risk to develop iron overload = 5% (about 1 in 20)
  - Chance for child of Caucasian proband to be at slightly increased risk for iron overload due to C282Y/H63D = 15% (about 1 in 7)

- Chance for child of Caucasian proband to be an HFE mutation carrier C282Y/WT = 80% (about 4 in 5)

- **H63D/H63D with elevated TS and ferritin**
  - Chance for sibling of Caucasian proband to be C282Y/C282Y and thus have at least a 50% risk to develop iron overload <1% (about 1 in 1000)
  - Chance for sibling of Caucasian proband to be at slightly increased risk for iron overload due to H63D/H63D = 30% (about 1 in 3); or to C282Y/H63D = 3% (about 1 in 30)
  - Chance for sibling of Caucasian proband to be an HFE mutation carrier C282Y/WT = 3% (about 1 in 40); or H63D/WT = 46% (about 1 in 2)
  - Chance for sibling of Caucasian proband to be a normal homozygote = 18% (about 1 in 5)
  - Chance for one or both parents of Caucasian proband to be at slightly increased risk for iron overload due to H63D/H63D = 16% (about 1 in 6)
  - Chance for one or both parents of Caucasian proband to be at slightly increased risk for iron overload due to C282Y/H63D = 11% (about 1 in 9)
  - Chance for one or both parents of Caucasian proband to be an H63D carrier = 98% (nearly all)
  - Chance for child of Caucasian proband to be at slightly increased risk for iron overload due to H63D/H63D = 15% (about 1 in 7), or to C282Y/H63D = 5% (about 1 in 20)
  - Chance for child of Caucasian proband to be an H63D carrier = 80% (about 4 in 5)
- **C282Y/H63D with iron overload**
  - Chance for sibling of Caucasian proband to be C282Y/C282Y and thus have at least a 50% risk to develop iron overload = 1.4% (about 1 in 70)
  - Chance for sibling of Caucasian proband to be at slightly increased risk for iron overload due to H63D/H63D = 4% (about 1 in 24); or to C282Y/H63D = 28% (about 2 in 7)
  - Chance for sibling of Caucasian proband to be an HFE mutation carrier C282Y/WT = 23% (about 1 in 4); or H63D/WT = 26% (about 1 in 4)
  - Chance for sibling of Caucasian proband to be a normal homozygote = 18% (about 1 in 5)
  - Chance for one or both parents of Caucasian proband to be C282Y/C282Y and thus have at least a 50% risk to develop iron overload = 2.8% (about 1 in 35)
  - Chance for one or both parents of Caucasian proband to be at slightly increased risk for iron overload due to H63D/H63D = 8% (about 1 in 12) or C282Y/H63D = 20% (about 1 in 5)
  - Chance for one or both parents of Caucasian proband to be an HFE mutation carrier C282Y/WT = 82% (about 4 in 5); or H63D/WT = 86% (about 9 in 10)
  - Chance for child of Caucasian proband to be C282Y/C282Y and thus have at least a 50% risk to develop iron overload = 3% (about 1 in 36)

- Chance for child of Caucasian proband to be at slightly increased risk for iron overload due to H63D/H63D = 7.5% (about 1 in 13), or to C282Y/H63D = 10% (about 1 in 10)
- Chance for child of Caucasian proband to be an an HFE mutation carrier C282Y/WT = 40% (about 2 in 5); or H63D/WT = 40% (about 2 in 5)

C282Y/WT with elevated TS and ferritin

- Chance for sibling of Caucasian proband to be C282Y/C282Y and thus have at least a 50% risk to develop iron overload = 1.4% (about 1 in 70)
- Chance for sibling of Caucasian proband to be at slightly increased risk for iron overload due to H63D/H63D = 1% (about 1 in 100); or to C282Y/H63D = 7% (about 1 in 16)
- Chance for sibling of Caucasian proband to be an HFE mutation carrier C282Y/WT = 44% (about 1 in 2); or H63D/WT = 12% (about 1 in 9)
- Chance for sibling of Caucasian proband to be a normal homozygote = 34% (about 1 in 3)
- Chance for one or both parents of Caucasian proband to be C282Y/C282Y and thus have at least a 50% risk to develop iron overload = 3% (about 1 in 33)
- Chance for one or both parents of Caucasian proband to be at slightly increased risk for iron overload due to C282Y/H63D = 15% (about 1 in 7)
- Chance for one or both parents of Caucasian proband to be an HFE mutation carrier C282Y/WT = 83% (about 5 in 6); or H63D/WT = 26% (about 1 in 4)
- Chance for one or both parents of Caucasian proband to be a normal homozygote = 69 (about 2 in 3)
- Chance for child of Caucasian proband to be C282Y/C282Y and thus have at least a 50% risk to develop iron overload = 2.7% (about 1 in 36)
- Chance for child of Caucasian proband to be at slightly increased risk for iron overload due to C282Y/H63D = 7.5% (about 1 in 13)
- Chance for child of Caucasian proband to be an HFE mutation carrier C282Y/WT = 43% (about 3 in 7; or H63D/WT = 7.5% (about 1 in 13)
- Chance for child of Caucasian proband to be a normal homozygote = 40% (about 2 in 5)

H63D/WT with elevated TS and ferritin

- Chance for sibling of Caucasian proband to be C282Y/C282Y and thus have at least a 50% risk to develop iron overload is negligible (<1% or <1/1000)
- Chance for sibling of Caucasian proband to be at slightly increased risk for iron overload due to H63D/H63D = 4.5% (about 1 in 22); or to C282Y/H63D = 3.2% (about 1 in 32)
- Chance for sibling of Caucasian proband to be an HFE mutation carrier C282Y/WT = 5% (about 1 in 20); or H63D/WT = 50% (about 1 in 2)
- Chance for sibling of Caucasian proband to be a normal homozygote = 37% (about 2 in 5)

- Chance for one or both parents of Caucasian proband to be at slightly increased risk for iron overload due to H63D/H63D = 9% (about 1 in 11) or C282Y/H63D = 6.6% (about 1 in 15)
- Chance for one or both parents of Caucasian proband to be an HFE mutation carrier C282Y/WT = 10% (about 1 in 10); or H63D/WT = 88% (about 9 in 10)
- Chance for one or both parents of Caucasian proband to be a normal homozygote = 74% (about 3 in 4)
- Chance for child of Caucasian proband to be at slightly increased risk for iron overload due to H63D/H63D = 7.7% (about 1 in 13) or C282Y/H63D = 2.6% (about 1 in 39)
- Chance for child of Caucasian proband to be an HFE mutation carrier C282Y/WT = 2.7% (about 1 in 36); or H63D/WT = 47% (about 1 in 2)
- Chance for child of Caucasian proband to be a normal homozygote = 41% (about 2 in 5)

WT/WT with elevated TS and ferritin

- Chance for sibling of Caucasian proband to be C282Y/C282Y and thus have at least a 50% risk to develop iron overload is negligible (<1% or <1/1000)
- Chance for sibling of Caucasian proband to be at slightly increased risk for iron overload due to H63D/H63D = 1.5% (about 1 in 65); or to C282Y/H63D = 1.1% (about 1 in 90)
- Chance for sibling of Caucasian proband to be an HFE mutation carrier C282Y/WT = 7.5% (about 1 in 13); or H63D/WT = 21% (about 1 in 5)
- Chance for sibling of Caucasian proband to be a normal homozygote = 69% (about 7 in 10)
- Chance for one or both parents of Caucasian proband to be an HFE mutation carrier C282Y/WT = 17% (about 1 in 6); or H63D/WT = 44% (about 3 in 7)
- Chance for one or both parents of Caucasian proband to be a normal homozygote = 88% (about 9 in 10)
- Chance for child of Caucasian proband to be an HFE mutation carrier C282Y/WT = 5.5% (about 1 in 18); or H63D/WT = 15% (about 1 in 7)
- Chance for child of Caucasian proband to be a normal homozygote = 80% (about 4 in 5)
- for non-Caucasian probands; risks to family members are not clear at this time

11. review of clinical recommendations

- (list what, if any, recommendations were made by physician doing CCE and that were reviewed during genetic counseling: ___
  ---

12. discussion of privacy and discrimination issues (review as indicated by level of subject's concern and screening test results)

- confidentiality of study data (computer and physical data safeguards; certificate of confidentiality)
- health insurance (US federal and state protections; increased risk with individual, non-group insurance; health advantages of early detection, etc.)
- life and disability insurance (fewer protections in US and Canada)
- employment discrimination (ADA protection for those in businesses with ≥15 employees; US protection for federal employees, CA and OR prohibition of use of genetic information for employment decisions)
- blood donation (not accepted in US at present; in Canada, healthy donors with HH eligible, but not more often than q. 56 days)

13. **exploration of psychosocial issues and assessment of subject's understanding of issues discussed** (discuss as indicated by genotype, degree of possible iron overload, or subject comments)

- how subject feels about test results (e.g., concerns about health, discrimination, stigmatization; feelings of diminished self-worth, anxiety, hopelessness, depression, guilt, blame, etc.)
- whom they intend to tell about test results (M.D.?, parents?, sibs?, kids?)
- what they intend to tell above individuals
- difficulties or reactions they expect or have encountered in telling others
- what they plan to do regarding the clinical recommendations (including perceived barriers to compliance)

14. **other information**

importance of completing questionnaires and other requirements of study participation

7. Ethical, Legal, Social Implications (ELSI) Study

7.1 Overview

The overarching aim of the ELSI research protocol is to provide data to assess the psychological and social effect of screening for hereditary hemochromatosis (HH) as it is implemented in the HEIRS study. In this, the ELSI studies are an integral part of the HEIRS study. The general questions of interest in the ELSI protocol are: (a) psychosocial impact of being screened for HH for all possible screening outcomes; (b) level of comprehension of information conveyed to participants about HH; (c) the extent of, attitudes toward, and reasons for sharing genetic risk information about HH with family members and others; (d) adherence to medical recommendations in regard to HH; and (e) evidence of stigmatization and discrimination in regard to screening for HH. These areas will be investigated through self-administered questionnaires. Baseline data will be collected on the Initial Screening form at the screening visit. All participants completing the Comprehensive Clinical Exam (CCE) will be mailed either the Post Result Form or, for family members of probands, the Family Post-Result form one week after notification of their exam results. A sample of participants not attending the CCE (described as non-CCE cases) will also be mailed the Post Result form one week after their Initial Screening (IS) results are mailed. One repeat mailing will be done, one month after the original mailing, if the subject does not respond to the first mailing. At Field Centers where response rates are still poor, a protocol will be developed to use telephone contact to increase response rates, allowing the participant to complete the form over the telephone if they request, and possibly adding incentives for completing the questionnaire. Participants who complete the Post Result forms will be mailed either the One Year Follow-up Form, or (for family members of probands) the Family One Year Follow-up Form one year after they receive their CCE results.

7.2 Guidelines and Procedures

The ELSI study instruments are: Initial Screening Form; Post Result Form; One Year Follow-Up Form; Family Post Result Form and the Family One Year Follow-Up. The Initial Screening Form is handed to the participant and completed during the initial screening visit. The Post Result Form and the One Year Follow-up Form are mailed to both the CCE ELSI and the Non-CCE ELSI participants. The Family Post Result Form and the Family One Year Follow-up Form are mailed to the family members (FMs) who have attended the family CCE. Both of the One Year Follow-up forms are mailed only to the participants who returned the Post Result Form. These instruments are mailed with stamped, self-addressed envelopes. All instruments are intended to be self-administered. However, some FCs with initially poor response rates have developed new procedures, such as completing the forms via a telephone interview format or offering incentives, so check with your local HEIRS staff for your FC's protocol. A passive, inclusive approach to literacy should be adopted. That is, those participants who request that the information be read to them should be provided with that help and will not be excluded from the study. It is expected, however, that most individuals with low levels of literacy will self-select out and not participate in the study. Because some

of the FCs are recruiting non-English speaking individuals, specifically, ones who may be monolingual in Spanish, in Vietnamese or Mandarin, all ELSI instruments will be translated into these three languages.

The follow-up forms mailed to participants after they receive their test initial screening (IS) results or CCE summary results are critical to assessing the ELSI impact of the study. CCE participants are mailed the Post Result Forms one week (plus or minus 2 business working days) after the CCE results letters are sent. The Non-CCE ELSI participants are mailed the Post Result forms one week after the IS results letters are sent. Non-CCE ELSI cases began to be identified beginning in February 2002, and are selected by the Coordinating Center (CoC), using an algorithym. Non-CCE ELSI cases will continue to be identified through October 7, 2002. Every Monday, the Field Centers (FCs) will receive an email to alert them to that the list of non-CCE ELSI cases identified for their site has been posted on the HEIRS website and on the local database. Approximately 15 non-CCE cases will be identified per site each week. The FCs should mail the Post Result Forms out to these participants each week, and should be mailed to them one week after their IS results letters are mailed. In both the CCE and the non-CCE cases, if the forms are not sent back within one month, the FCs should send out one more mailing, including a reminder letter and a new copy of the form. Protocols regarding incentives and telephone interviews may differ by FC, so local procedures should be ascertained. The website tracking system will alert the FCs with the dates that the repeat mailings should be made. It is crucial for the FCs to data enter the date that the results letters are sent out in the tracking system so it can calculate the dates for the subsequent mailings. It is also important to enter the dates that the forms are received back at the FCs, so that the tracking system will not alert the sites for the repeat mailings that are not needed.

The One Year Follow-up Forms are mailed only to participants who returned the Post Result Form. This includes Non-CCE ELSI participants, CCE participants, control CCE participants and family study CCE participants. FCs should query the tracking system on the website each week to determine which participants are due to be sent a One Year Follow-up Form that week. FCs should then send a second mailing after a month if there has been no response to the first mailing. The website can be queried periodically (once a week is recommended) to find participants who are due to receive this one month mailing. FCs may also develop a protocol for offering incentives or follow-up by telephone.

The ELSI cover letters and mailing labels can be downloaded and printed through the Field Centers' local database. All of the ELSI forms are printed and shipped through the CoC, and should be on hand and ready at each Field Center. Assemble the appropriate Post Result Form and corresponding instructions, with a stamped, self-addressed envelope for returning the form, again taking care that the appropriate forms are mailed for family and main-study participants. Envelopes for participants in the main study and the family study should be mailed one week (plus or minus 2 working days) following notification of their test results from the CCE. For the non-CCE cases, the same forms

and envelopes should be mailed one week (plus or minus 2 working days) after the initial screening results letters have been mailed.

7.2.2 Data Entry

Once any completed forms are received at the FC, its data should be entered into the HEIRS website, via the data entry menu. The data entry screen will reflect the actual form itself. Responses should be entered exactly as they are recorded on the participant's form. The only exception to this is in question #28 of the Post Result Form and the Family Post Result Form. If the participant gives the response "retired" to this employment question, leave this field blank, as a missing data field.

7.2.3 HEIRS Website Tracking System

The CoC has developed a CCE and ELSI tracking system on the HEIRS website, . It can be accessed under the "Data" menu, by choosing "Tracking System". This will track both the data and the response rates for the CCE and non-CCE ELSI forms mailings. The tracking system includes the date the participant was identified by the CoC as CCE eligible (CCE ELSI cases), the date the CCE was scheduled, whether the CCE was refused and if so why, the date the CCE was performed, the date the Post Results forms should be sent, the date the Post Results forms were sent, the date the returned Post Results forms were received, and the date that the a repeat mailing should be made if the Post Results forms were never returned. The same process applies to the One Year Follow-up Forms. A report is available that tracks both the CCE ELSI and the non-CCE ELSI cases. Since this tracking system will alert the FCs as to the dates that any repeat mailings should be made, the date the results letters (both IS and CCE results letters) were sent out must be data entered to calculate these dates, as well as the dates that the forms were received back at the FC. This tracking system also records the reasons for the CCE refusal, as well as the retention into the CCE. Various website reports will be generated based upon this tracking system, so the importance of the timely data entry is stressed.

The following is a list of the Tracking Reports currently available on the website (as of December 2002) on the website:

Tracking Reports

- Post Result Mailings Due
- Post Result Follow-up Mailings Due
- CCE Status
- ELSI Case Status
- ELSI Study Summary
- Study CCE Summary
- CCE Refusal Reasons
- Provisional Case Status
- Quantitative Phlebotomy / Liver Biopsy Follow-up
- ELSI - Post Result Returned by Letter Type

ELSI - Cases Identified by Week
Control Status
Control Status Muldrow Form
1 Year Follow-up Mailings Due
1 Year Follow-up 1 Month Follow-up Mailings Due
Recruitment by Race and Clinic
Recruitment by Race and Clinic - V2
CCE Data Entry Forms

The Field Center will use the tracking system on the website to record in the database the date the results letter was sent in the database.

- One week later, a reminder, a stamped, self-addressed envelope, and the Post-Result Form will be generated and mailed.
- The actual date of mailing will then be recorded in the tracking system.
- If the form is not returned within one month, another reminder, SASE, and Post-Result Form will be generated and mailed.
- The actual date of this mailing will then also be recorded in the tracking system.
- Whenever the Post-Result Form is returned, the data will be entered.

The same process should be used for to the One Year Follow-up Form.

The Family Post Result Form and Family One Year Follow-up Form will utilize the same process. The website tracking system and reports for the family study will be developed when the family study begins.

7.3 FORMS

7.3.1 Post Result Form

INSTRUCTIONS FOR COMPLETING:

Staff will complete the header information at the top of the first page of the form (participant ID and acrostic) before it is mailed. Staff will also complete the acrostic on the top of each subsequent page. The participant will complete the remainder of the form and will mail it back.

1. How much you used each of the following sources for more information about hemochromatosis and iron overload (1a-1f)? Participant checks the appropriate box for each question. Check only one box per question. Other sources used for information may be written in the comment box (1f).
2. How you were first notified of results and how you felt (2-2a). Check only one box per each statement.
3. Opinion of information received about hemochromatosis and test results (3a-3c). Check only one box per each statement.

4. Genetic testing to find out about disease risk is good idea. Check only one box per statement.
5. Statements about test results received (5a-5f). Check only one box per each statement.
6. What you were told to do in response to test results (6a-6e). Check only one box per each statement.
7. Your belief that recommendations will help health. Check only one box.
8. Your confidence that you can follow recommendations. Check only one box.
9. How often you have experienced various feelings since receiving test results (9a-9m). Check only one box per each statement.
10. Belief that person's genetic risk should be shared with family members. Check only one box. If believe information should be shared, how (10a-10c)? Check only one box per statement.
11. Person you are likely to share information with (11a-11h). Check only one box per statement.
12. Encouragement of others to be tested for hemochromatosis (12a-12g). Check only one box per statement.
13. Possible reasons why you think people get sick (13a-13e). Participant checks the appropriate box for each statement. Check only one box per statement.
14. Status of general health. Participant rates his/her health status. Participant checks the appropriate box.
15. How true or false are the following statements (15a-15d) regarding your health? Participant checks the appropriate box for each statement. Check only one box per statement.
16. How you feel since receiving the test results (16a-16e). Participant checks the appropriate box for each question. Check only one box per question.
17. Rank how serious hemochromatosis is to other medical conditions. Participant checks the appropriate box.
18. Problems associated with hemochromatosis or iron overload (18a-18i). Participant checks only one box per statement (yes, no, or don't know).
19. True and false questions about hemochromatosis (19a-19p). Participant checks the appropriate box for each question. Check only one box per statement.
20. Genetic testing is good because (20a-20h). Participant checks the appropriate box for each statement. Check only one box per each statement.
21. Possible reasons why genetic testing is not good (20i-20o)– Participant checks the appropriate box for each statement. Check only one box per each statement.
22. Opinions and practices in regard to health care in general (21-25). Participant checks the appropriate box for each statement. Check only one box per each statement.
23. Type of insurance you have (if any) (26a-26c). Participant checks the appropriate box for each statement. Check only one box per each statement.
24. If you have health insurance, who pays most of the cost (27)? Participant checks the appropriate box.
25. Employment status (28). Participant checks the appropriate box. If the person writes in "retired", please leave this blank as a missing field in data entry.

26. Highest level of education completed (29). Participant checks the appropriate box.

END OF FORM

7.3.2 Family Post Result Form

INSTRUCTIONS FOR COMPLETING FORM:

Staff will complete the header information at the top of the first page of the form (participant id and acrostic) before it is mailed. Staff will also complete the acrostic on the top of each subsequent page before it is mailed. The participant will complete the remainder of the form and mail it back.

1. How you found out about study. Participant checks the appropriate sources/reason(s) to indicate how heard about the study.
2. How much you knew about hemochromatosis before contacted about being in study. Participant checks only one box.
3. How much you used each of the following sources for more information about hemochromatosis/iron overload (3a-3f) – Participant checks the appropriate box for each question. Check only one box per question. Other sources used for information may be written in the comment box (1f).
4. Knowledge of hemochromatosis /iron overload in family before the study. Participant checks only one box.
5. Knowledge of family members who had to donate blood regularly for their health before this study. Participant checks only one box.
6. Opinion of information received about hemochromatosis and test results (6a-6c). Check only one box per each statement.
7. You're level of agreement that in general genetic testing is good idea to find out about disease risk. Check only one box per statement.
8. Statements about test results received (8a-8f). Check only one box per each statement.
9. What you were told to do in response to test results (9a-9e). Check only one box per each statement.
10. Belief that recommendations will help health. Check only one box.
11. Confidence that can follow recommendations. Check only one box.
12. How much you have experienced various feelings since receiving test results about possible risk of hemochromatosis/iron overload in family (12a-12m). Check only one box per each statement.
13. Belief that person's genetic risk should be shared with family members. Check only one box. If believe information should be shared, how (13a-13c)? Check only one box per statement.
14. Whom you are most likely to share information with (14a-14h). Check only one box per statement.
15. Encouragement of others to be tested (15a-15g). Check only one box per statement.

16. Possible reasons why you think people get sick (16a-16e). Participant checks the appropriate box for each statement. Check only one box per statement.
17. Status of general health. Participant rates his/her health status. Participant checks the appropriate box.
18. How true or false are the following statements (18a-18d) regarding your health? Participant checks the appropriate box for each statement. Check only one box per statement.
19. How you feel since receiving the test results (19a-19e). Participant checks the appropriate box for each question. Check only one box per question.
20. Rank how serious hemochromatosis is to other medical conditions. Participant checks the appropriate box.
21. Genetic testing is good because (21a-21h). Participant checks the appropriate box for each statement. Check only one box per each statement.
    Possible reasons why genetic testing is not good (21i-21o)– Participant checks the appropriate box for each statement. Check only one box per each statement.

22-26. Opinions and practices in regard to health care in general (22-26) Participant checks the appropriate box for each statement. Check only one box per each statement. .

27. Type of insurance you have (if any) (27a-27c). Participant checks the appropriate box for each statement.
28. If you have health insurance, who pays most of the cost (28). Participant checks the appropriate box.
29. Employment status (29). Participant checks the appropriate box. If the participant writes in “retired”, please do not data enter anything. Leave this item blank as a missing data filed.

END OF FORM

7.3.3 One Year Follow-Up Form

INSTRUCTIONS FOR COMPLETING FORM:

Staff will complete the header information at the top of the first page, to include Participant id and acrostic. Staff also completes the acrostic at the top of each page. Once completed, the form is mailed. The participant will complete the remainder of the form and mail it back.

1. How much you have used each of the following sources for more information about hemochromatosis and iron overload in the past year (1a-1f). Participant checks the appropriate box for each question. The answers are none, some or a lot. Check only one box per question. Other sources used for information may be written in the comment box (1f).
2. Opinion of information received about hemochromatosis and test results in the beginning of the study (2a-2c). The answers are strongly agree, agree, disagree and strongly disagree. Check only one box per each statement.

3. Genetic testing is good idea to find out about disease risk. Check only one box per statement.
4. Statements about test results received (4a-4f). Check only one box per each statement.
5. What to do in response to test results (5a-5e). Check only one box per each statement.
6. Followed the recommendations given regarding hemochromatosis/iron overload. Check only one box.
7. Belief that recommendations are helping your health. Check only one box.
8. How much you have experienced various feelings in the past several months (8a-8m). Check only one box per each statement.
9. Belief that person's genetic risk should be shared with family members. Check only one box. If believe information should be shared, how (9a-9c)? Check only one box per statement.
10. Whom you have shared information with about risk for iron overload in the past year (10a-10h). Check only one box per statement.
11. If shared information about risk with any family members, why (11a-11g). Participant checks the appropriate box for each question. Check only one box per statement.
12. If did not share information about risk with family members, why (12a-12j). Participant checks the appropriate box for each question. Check only one box per statement.
13. Encouragement of others to be tested for risk since received your results (13a-13g). Participant checks the appropriate box for each question. Check only one box per statement.
14. Possible reasons why you think people get sick (14a-14e). Participant checks the appropriate box for each statement. Check only one box per statement.
15. Status of general health. Participant rates his/her health status. Participant checks the appropriate box.
16. How true or false are the following statements (16a-16d) regarding your health? Participant checks the appropriate box for each statement. Check only one box per statement.
17. How you feel since receiving the test results in the past year (17a-17e). Participant checks the appropriate box for each question. Check only one box per question.
18. Rank how serious hemochromatosis is to other medical conditions. Participant checks the appropriate box.
19. Problems associated with hemochromatosis or iron overload (19a-19i). Participant checks only one box per statement.
20. True and false questions about hemochromatosis (20a-20p). Participant checks the appropriate box for each question. Check only one box per statement.
21. Genetic testing is good because (21a-21h). Participant checks the appropriate box for each statement. Check only one box per each statement.
22. Possible reasons why genetic testing is not good (21i-21o)– Participant checks the appropriate box for each statement. Check only one box per each statement.

23. In the past year any problems finding/keeping a job or getting a raise/promotion (22). Participant checks the appropriate box for each statement. If answer yes, go onto next question about relation to hemochromatosis/iron overload and mark one box.
24. In the past year been turned down as a volunteer for free blood donation (23). Participant checks the appropriate box for each statement. If answer yes, go onto next question about relation to hemochromatosis/iron overload and mark one box.
25. In the past year have you had an insurance policy canceled or trouble getting/keeping/increasing the amount (24a-24c). If answer yes, go onto next question about relation to hemochromatosis/iron overload and mark one box.

END OF FORM

7.3.4 Family One Year Follow-Up Form

INSTRUCTIONS FOR COMPLETING FORM:

Staff will complete the header information at the top of the first page, to include Participant id and acrostic. Staff also completes the acrostic at the top of each page. Once completed, the form is mailed. The participant will complete the remainder of the form and mail it back

1. How much you have used each of the following sources for more information in the past year about hemochromatosis and iron overload (1a-1f). Participant checks the appropriate box for each question. Check only one box per question. Other sources used for information may be written in the comment box (1f).
2. Opinion of information received about hemochromatosis and test results in the beginning of the study (2a-2c). Check only one box per each statement.
3. Genetic testing is good idea to find out about disease risk. Check only one box per statement.
4. Statements about test results received (4a-4f). Check only one box per each statement.
5. What told to do in response to test results (5a-5e). Check only one box per each statement.
6. Followed the recommendations given regarding hemochromatosis/iron overload. Check only one box.
7. Belief that recommendations are helping your health. Check only one box.
8. How much you have experienced various feelings in the past several months (8a-8m). Check only one box per each statement.
9. Belief that person's genetic risk should be shared with family members. Check only one box. If believe information should be shared, how (9a-9c)? Check only one box per statement.
10. Whom you have shared information with about risk for iron overload (10a-10h). Check only one box per statement.

11. If shared information about risk with any family members, why (11a-11g). Participant checks the appropriate box for each question. Check only one box per statement.
12. If did not share information about risk with family members, why (12a-12j). Participant checks the appropriate box for each question. Check only one box per statement.
13. Encouragement of others to be tested for risk since received your results (13a-13g). Participant checks the appropriate box for each question. Check only one box per statement.
14. Possible reasons why you think people get sick (14a-14e). Participant checks the appropriate box for each statement. Check only one box per statement.
15. Status of general health. Participant rates his/her health status. Participant checks the appropriate box.
16. How true or false are the following statements (16a-16d) regarding your health? Participant checks the appropriate box for each statement. Check only one box per statement.
17. How you feel since receiving the test results in the past year (17a-17e)? Participant checks the appropriate box for each question. Check only one box per question.
18. Rank how serious hemochromatosis is to other medical conditions. Participant checks the appropriate box.
19. Genetic testing is good because (19a-19h). Participant checks the appropriate box for each statement. Check only one box per each statement.
    Possible reasons why genetic testing is not good (19i-19o)– Participant checks the appropriate box for each statement. Check only one box per each statement.
20. In the past year any problems finding/keeping a job or getting a raise/promotion (20). Participant checks the appropriate box for each statement. If answer yes, go onto next question about relation to hemochromatosis/iron overload and mark one box.
21. In the past year been turned down as a volunteer for free blood donation (21). Participant checks the appropriate box for each statement. If answer yes, go onto next question about relation to hemochromatosis/iron overload and mark one box.
22. In the past year have you had an insurance policy canceled or trouble getting/keeping/increasing the amount (22a-22c). If answer yes, go onto next question about relation to hemochromatosis/iron overload and mark one box.

END OF FORM

7.4 Definition of ELSI Cases and Controls

Table 1 describes the ELSI cases and controls. The ELSI protocol will seek subjects in all of these categories. The numbers of available subjects in each cell is an estimate; the actual number will not be known until the study is underway.

TABLE I

ELSI CASES (including CCE cases) AND CONTROLS

IRON LEVELS MEETING CCE THRESHHOLD	IRON LEVELS BELOW CCE THRESHHOLD
GROUP #1 C282Y/C282Y (available N ≈ 201)	GROUP #7 C282Y/C282Y (available N≈109)
GROUP #2 C282Y/H63D (available N ≈ 58)	GROUP #8 (Letter 3) C282Y/H63D H63D/H63D C282Y/+ (available N ≈ 8,617) N=120
GROUP #3 H63D/H63D (available N ≈ 19)	GROUP #9 (Letter 3a) C282Y/H63D H63D/H63D C282Y/+ All with Alert Values (available N ≈ ?) N=120
GROUP #4 C282Y/+ (available N ≈238)	GROUP #10 (Letter 4) H63D/+ (available N ≈ 12, 493) N=120
GROUP #5 H63D/+ (available N ≈ 151)	GROUP #11 (Letter 4a) H63D/+ With Alert Value (available N ≈ ?) N=120
GROUP #6 +/+ (available N ≈ 716)	GROUP #12 (Letter 2) +/+ (available N > 93,000) N≈2000 CCE and 200 non-CCE
	GROUP #13 (Letter 2a) +/+ With Alert Value (available N = ?) N=120

Groups 1, 2, 3, 4, 5, 6, and 7 will be eligible for the comprehensive clinical exam. Clearly some of these cells are underpowered. If adequate numbers of participants prove not to exist, cells will be collapsed for analysis. Specifically, the boxes representing groups 2, 3, 4, and 5 may have to be collapsed.

Groups 8, 9, 10, 11, and 13 will only be ELSI cases. For these groups, subjects will be selected only if they have provided the following information on the baseline screening form: gender, birthdate, race or ethnicity, health status, and psychological health. Eligible subjects will be selected until the indicated numbers of initial responses are

received for each group. These subjects will be selected in a fashion that produces approximately equal numbers of non-CCE cases in each Field Center.

Group 12 will constitute controls for the ELSI study, with separate controls for each part of the ELSI study. The CCE controls will be the same for the ELSI and the main part of the study. The controls for the non-CCE ELSI study will be selected the same way as Groups 8-11 and 13, and will be frequency-matched to these groups as a whole with respect to age, gender, and ethnicity.

> **NOTE**: An HEIRS participant does not become an ELSI case until s/he returns the ELSI form. Therefore, the number of Post Result forms that need to be mailed out, and followed up, may differ by Field Center and will, in any case, be larger than the proposed sample size of 800. Currently, the Steering Committee has approved mailing ELSI forms to approximately 2250 non-CCE ELSI cases, or 450 per Field Center.

7.5 Study Flow

As participants are recruited, they will be given the Initial Screening Form. Half of the information on this form constitutes baseline psychosocial data for the ELSI protocol. This form will be filled out by all 100,000 participants. After the screening tests have been completed, all 100,000 participants will be informed of their screening test. Those who screen positive, plus a frequency matched set of controls, will be invited to the comprehensive clinical exam.

Of the remaining participants, approximately 2250 will be ELSI cases who will not undergo the comprehensive clinical exam. Selection of potential ELSI participants will be done at the Coordinating Center. To be eligible as an ELSI participant, a minimum data set must be completed on the Initial Screening Form. The questions in this minimal data set are listed below:

- Gender
- Birth date
- Race/Ethnicity
- Item on health status (1 response required) or true/false questions on perceived health (at least 2 of 4 subquestions required)
- Psychological health question (at least 50% of responses filled in)

> **NOTE**: Any participant who answers *either* the “race” or the “ethnicity” question should be considered to have completed that data point.

Approximately one week after receiving their screening results by letter, those participants who are ELSI cases but not invited to the CCE, will receive the Post-Result Form in the mail with instructions to fill it out and return it in the enclosed, stamped, self-addressed envelope. Approximately one week after receiving their CCE results, by

phone or by mail, CCE participants will receive the Post Result Form in the mail with instructions to fill it out and return it in the enclosed, stamped, self-addressed envelope.

If the form is not returned after the follow-up mailing, the participant will be dropped from consideration as an ELSI participant. However, Field Centers with low response rates may make extra efforts to contact non-responding subjects by phone, either to remind them to send in these forms, or to complete these forms over the phone in an interview format. Some Field Centers are including incentives to complete the form, such as phone cards or Wal-Mart gift certificates. Please check with your PI or HEIRS staff for procedures at your Field Center.

One year after the Post Result Form is returned, all ELSI cases and controls will receive the One Year Follow-Up. Only those participants who return the Post Result Form will be sent the One Year Follow-up Form. All procedures, including the second, reminder mailing and possible incentives or phone contact will follow the protocol of the Post Result Form.

7.6 Substudies

Two ELSI substudies will be performed. These are described in Appendices K and L.

8. FOLLOW-UP OF QUANTITATIVE PHLEBOTOMY, LIVER BIOPSIES AND ANNUAL CLINICAL FOLLOW-UPS

8.1 Overview

Phlebotomy is the preferred treatment for iron overload of diverse etiologies, and is safe, effective, efficient, and economical. In some cases, phlebotomy yields a measurement of iron stores which can confirm (or exclude) the diagnosis of iron overload. Performance of phlebotomy is not part of the HEIRS study. However, the HEIRS study will attempt to capture the quantitative phlebotomy (qph) data from the participants who are C282Y homozygotes or have confirmed elevations of Transferrin Saturation (TS) and Serum Ferritin (SF) upon completion of the Comprehensive Clinical Exam (CCE). Beginning in June 2003, the study will fund the central reading of all liver biopsy samples (see section 8.8.4). Prior to this, the study will attempt to capture liver biopsy data directly from pathology reports on these same participants. The HEIRS study will follow the participants for this data beginning with the date of the Initial Screening visit.

8.2 Subject Selection

The CoC will identify the participants that are C282Y homozygotes or have confirmed elevations of TS and SF after the CCE. The Coordinating Center (CoC) will provide this list of participants to the Field Centers (FCs) for them to follow for quantitative phlebotomy (qph) and/or liver biopsies. This list is on the HEIRS website, under the "Tracking" menu, and is called "Quantitative Phlebotomy/Liver Biopsy Follow-up." The list includes the participant id, acrostic, date of initial screen consent and the date of the CCE. Each field center will only see the list that pertains to their site. In some situations, Field Center staff may be aware of CCE participants not on this list that are receiving quantitative phlebotomy, other diagnostic procedures or treatment for iron overload. Information on the follow-up diagnosis and/or treatment for these participants should also be collected if possible.

8.3 Serum Ferritin and Transferrin Saturation Criteria for Initiating Phlebotomy

It is standardly recommended that phlebotomy be initiated in men and post-menopausal women with serum ferritin greater than 300 ng/mL (300 ug/L), and in non-pregnant women in reproductive years with serum ferritin greater than 200 ng/mL (200 ug/L). The HEIRS study will utilize these same serum ferritin lab values, as well as transferrin saturation (males, >50, females >45), for use in recommending phlebotomy to the HEIRS participants. In pregnant women, phlebotomy therapy is recommended if serum ferritin greater 500 ng/mL (500 ug/L) and if significant cardiac or hepatic dysfunction attributable to iron overload is present; otherwise, phlebotomy therapy should be started after the conclusion of pregnancy.

8.4. Phlebotomy Procedures

Phlebotomy is not part of the HEIRS study procedures. Phlebotomy should be conducted by experienced persons and supervised by a physician. The HEIRS study does not mandate that the phlebotomy be performed by the HEIRS personnel, in fact, many of the treatments will be performed by the participant's primary physician, or referral. There are a few field centers that will be performing the phlebotomy locally. Persons undergoing phlebotomy should be encouraged to maintain adequate hydration before and after phlebotomy, and to avoid vigorous physical activity for 24 hours after phlebotomy. It is recommended that phlebotomy be performed using a 19 - 21-gauge needle (butterfly) and upper extremity venipuncture. Blood is collected via a tubing set into a transfusion collection bag or an evacuated bottle weekly to permit measurement of the volume removed. The transfusion bag or evacuated bottle will be weighed and standardized, for use in recording at each treatment session (see Section 8.12). The blood removed should be weighed at each treatment session. (see Section 8.8.1 below). This is accomplished by weighing the empty container, then weighing the container with the blood removed present, and then subtracting the empty container weight from the weight of the container with the blood removed present. This is captured on the Treatment Session Form. This form also records the most current (within 2 weeks) hematocrit and hemoglobin values. Once this form is data entered, then the CoC will calculate the amount or iron removed as well. It is possible to remove about 1 unit (450 - 500 mL) weekly in some men and persons with large body mass. **Please be sure to verify the exact weight used for declaring 1 unit at your FC; some centers will be 450mL and some use 500 mL. Be sure to notify the CoC as to which weight is a standard unit at your site.** In some women, persons with small body mass, elderly persons, and patients with co-existing anemia, cardiac problems, or pulmonary problems, the removal of about 0.5 unit weekly is more appropriate. Phlebotomy alone is a stimulus for erythropoietin production and release; erythropoietin therapy should be reserved for patients with renal dysfunction or anemia of chronic disease. Implantation of venous access devices solely for the purpose of facilitating phlebotomy should be performed only if absolutely necessary.

8.4.1. Monitoring Phlebotomy

All available serum ferritin values associated with a phlebotomy session should be recorded on the Treatment Session form. Serum ferritin concentration is the most reliable, readily available, and inexpensive means to assess iron stores and the progress of therapy in most patients. The HEIRS study will utilize the serum ferritin values to monitor the phlebotomy treatments, and also will measure the blood removed from each treatment. In persons whose serum ferritin concentration is extremely high at diagnosis, measurement of serum ferritin monthly during phlebotomy therapy is usually adequate. It is recommended that the serum ferritin be quantified with each one or two treatments in all persons undergoing phlebotomy once the serum ferritin is less than 100 ug/L. It must be noted that the quantitative phlebotomy is done at the discretion of the treating physician. Guidance for physicians to treat HEIRS participants can be obtained on the public section of the HEIRS website. HEIRS personnel should be available to answer questions by non-HEIRS physicians regarding HEIRS participants.

8.4.2. Iron Depletion Endpoint

Once the participant has reached iron depletion and stopped quantitative phlebotomy, the HEIRS study does not require any other follow-up information, except for the Annual Clinical Follow-up Form, both the Participant and Physican versions (see Section 8.10.1 and 8.10.2). The information that the participant has achieved iron depletion or other reasons for stopping phlebotomy will be captured on the Monthly Quantitative Phlebotomy Summary Form (Section 8.8.2).

For the study definition of iron removed by phlebotomy, the definition of iron depletion below will be used. It is also recommended that phlebotomy, for the purposes of inducing iron depletion, be discontinued when the serum ferritin concentration is less than 20 ng/mL (20 ug/L), when the hemoglobin concentration is less than 11.0 g/dL (110 g/L), or when the hematocrit is less than 33.0% (0.33) for more than three weeks (in patients without chronic anemia).

8.5. Potential Adverse Effects of Phlebotomy

The participants will be encouraged to maintain diaries to record any information regarding side effects of the phlebotomy. These diaries are used at each Field Center's discretion. The diaries are not data entered into the web site. Complications of phlebotomy include local discomfort or bruising at site of venipuncture, diaphoresis (excessive sweating), weakness, tachycardia, loss of consciousness, and postural hypotension. These side effects will be captured on the Treatment Session Form (Section 8.8.1.), which is filled out by the clinical or HEIRS staff .

8. 6. Compliance with Phlebotomy

Most persons comply with recommendations that they undergo phlebotomy after an appropriate explanation of iron overload and phlebotomy is made. However, there are several circumstances in which persons do not agree to undergo phlebotomy, or which are associated with poor compliance with a recommended course of phlebotomy. These include: physical discomfort, intolerance, or other complaints about phlebotomy; intercurrent illness; monetary or insurance problems related to phlebotomy or iron overload; interference with work due to phlebotomy; perception of value of phlebotomy or seriousness of iron overload; and re-location such that healthcare professionals who are skilled in phlebotomy for iron overload are not readily available. These reasons will be captured on the HEIRS Monthly Quantitative Phlebotomy Summary Form (Section 8.8.2).

If participants initially decline the phlebotomy or liver biopsy, the FC should still periodically (every 3 months or at the FC discretion) review the records to ascertain if the participant has changed their mind. This may occur for many reasons, such as participant being overwhelmed or uneducated as to the procedures.

8.7. Quantitative Phlebotomy and/or Liver Biopsy Data Collection

HEIRS staff will be responsible for acquiring the data on each participant undergoing therapeutic phlebotomy. This will also include gathering the data from the other clinics where the quantitative phlebotomy is being performed. This data will be abstracted from two sources. First, at each clinic where quantitative phlebotomy is being performed on HEIRS participants, a notebook containing data sheets for each participant will be maintained. These sheets will include entries for the data items in 8.8.1 and 8.8.2 below. These forms will need to be explained to the phlebotomy clinics by the HEIRS personnel, and the forms will need to be inventoried and maintained by the HEIRS staff. Outside phlebotomy center personnel will be asked to maintain these records, along with the HEIRS staff's assistance. The HEIRS staff is responsible for monitoring quantitative phlebotomies and reviewing the notebooks monthly for use in the Monthly Summary Forms. The HEIRS staff will also attempt to retrieve any missing data using participant medical records, and abstract the weekly treatment data for use in filling out the Treatment Session Form, and data entry into the central study database. The second source of data collection is abstraction from electronic medical records where available.

The CoC will provide a list of participants to the Field Centers (FCs) for them to follow for quantitative phlebotomy and/or liver biopsies. This list is on the HEIRS website, under the "Tracking", menu, and is called "Quantitative Phlebotomy/Liver Biopsy Follow-up." The list includes the participant id, acrostic, date of initial screen consent and the date of the CCE. Each field center will only see the list that pertains to their site. In some situations, Field Center staff may be aware of CCE participants not on this list that are receiving quantitative phlebotomy or other diagnostic procedures or treatment for iron overload. Information on the follow-up diagnosis and/or treatment for these participants should also be collected if possible.

A web site report will assist in checking ongoing quantitative phlebotomy records that have not been checked in the past 90 days. The report is in the "Report" menu, then "Quantitative Phlebotomy Reports" and is called "Ongoing qph participants not checked in Last 90 days". The menu is copied below. This report will alert you to how many ongoing qph pts that have not been checked in the last 90 days. If you click on your field center, there is a drop down box that will list the ids of those participants not checked.

QUANTITATIVE PHLEBOTOMY MENU

? Phlebotomy Treatments by Field Center
? Initial Screen vs. Phlebotomy Treatments
? Count and Proportion of Provisional Cases that have a Monthly Phlebotomy Form by Field Center
? Quantitative Phlebotomy Participants Not Checked in Last 90 Days
? Quantitative Phlebotomy Participants by Race/Ethnicity
? Quantitative Phlebotomy Participants by CCE Result Status
? Quantitative Phlebotomy Participants by HFE Genotype
? Quantitative Phlebotomy Participants by Race/Ethnicity and CCE Status
? Quantitative Phlebotomy Participants by Race/Ethnicity and HFE Genotype
? Quantitative Phlebotomy Participants by CCE Status and HFE Genotype

Data should also be collected on participants who have had liver biopsies since the date of Initial Screening. Prior to June 2003, FC staff should obtain the pathology report on any participant who has had a liver biopsy. This pathology report is then abstracted onto the Liver Biopsy Pathology Report Summary Form. For questions concerning the data abstraction, FCs are advised to discuss them with their local Adjudication Subcommittee representative. After June 2003, liver biopsies on HEIRS participants will be read centrally.

Field Centers are responsible for obtaining the follow-up clinical data on the identified participants and recording the information in the following forms:

- Therapeutic Phlebotomy Form- Treatment Session Form (see section 8.8.1 below)
- Monthly Quantitative Phlebotomy Treatment (see section 8.8.2. below)
- Liver Biopsy Pathology Report Summary Form (see Section 8.9.3. below)
- Central Reading Liver Biopsy Form (see Section 8.9.4. below)
- Annual Clinical Follow-up Form -Participant and Clinician Versions (see Sections 8.10.1. and 8.10.2. below)

8.7.1. Recommended Elements and Procedures for the Data Collection

The determination of the quantity of iron removed by therapeutic phlebotomy will be done centrally through the CoC, based on a formula involving the volume of blood removed and a recent hematocrit or hemoglobin value . It must be noted that this **is not** done at the FCs for HEIRS purposes. The cumulative total will be computed as the sum of the product of these measures from each phlebotomy session up until iron depletion is reached. Since both the volume of blood removed and hematocrit can change from week to week, values at each week are preferred. However, to achieve a balance between the cost of data

collection and completeness of data, these weekly data will be collected monthly. The approximate amount of iron removed (mg) =

current hematocrit (percent) x blood removed (mL)
or
current hemoglobin (g/dL) x blood removed (mL) x 0.0347.
(one unit of blood is approximately equivalent to 200 mg of iron).

The required elements for data collection are included in the following sections.

8.8. QUANTITATIVE PHLEBOTOMY AND LIVER BIOPSY FORMS -QUESTION BY QUESTION

8.8.1 Therapeutic Phlebotomy Form- Treatment Session Form

INSTRUCTIONS

Staff will complete the header at the top of the page, to include:

- Participant id and acrostic;
- Date that the form was filled out;
- Completed by: assigned digitial code or initials;
- The name of the clinic or facility where the phlebotomy was performed;
- Record the visit number of this treatment, out of the number of treatments for that month (e.g., treatment # 1 of 2 for the month, 2 of 4, etc.)

Please complete the following information about the treatment, obtained from the records where the phlebotomy was performed, or from the electronic medical records (where applicable):

- #1. The date of the phlebotomy treatment that is being recorded
- #2. Please record whether the blood was weighed, by checking the yes or no box.
  - If no is checked, skip down to the volume of blood that was removed, and record the number of units that was removed. Be sure to record exactly what was in the chart. This also pertains to any fractionated amounts, such as .5 or .75 units. See Section 8.4 for weights in units recorded as 450 ml or 500 ml.
  - If yes is checked, please record the weight of the empty container (should be previously standardized, in grams), then the weight of the container with the blood removed, then calculate the weight of the blood removed only (all in grams). This is calculated by subtracting the weight of the empty container only from the weight of the container with the blood removed.
- #3. Record the most recent hematocrit (current or within 2 weeks) and the date if different than the treatment date;
- #4. Record the most recent hemoglobin (current or within 2 weeks) and the date if different than the treatment date;

- #5. Record the serum ferritin and the date if different than the treatment date.
- #6-#14. Record any and all adverse effects that have been noted, either by the participant on the diaries or noted in the chart. Be sure to record exactly what was in the chart or the diaries. Please place a check mark in all of the boxes that apply. These include local discomfort or bruising at site of venipuncture, diaphoresis (excessive sweating), weakness, tachycardia, loss of consciousness, and postural hypotension. If no side effects were noted, then check the "None experienced" box.
- Any additional comments may be written in at the bottom of the form under the "Comments" section.

End of Form

8.8.2 HEIRS Monthly Quantitative Phlebotomy Treatment Summary

INSTRUCTIONS

Staff will complete the header at the top of the page, to include:

- Participant id and acrostic
- Date that the form was filled out
- Completed by: assigned digitial code or initials

Please complete the following information about the treatment, obtained from the records where the phlebotomy was performed, or from the electronic medical records (where applicable):

- Is phebotomy being performed at least once a month? Check the yes or no box. If the response is “No”, then access to the Treatment Session Form for data entry is denied.
- If the phlebotomy has not started, has stopped, or is being less than once a month, indicate the reason (no visit found related to iron overload, therapeutic phlebotomy is not indicated, iron depletion has been reached and now on maintenance phlebotomy, complication or side effect, illness, patient has, or believes monetary or insurance problems are related to the phlebotomy, phlebotomy is interferring with work or work schedule, patient believes iron overload is not serious or important, patient moved or left health plan, patient deceased, not known, or other reasons that are specified).
  Be sure to record exactly what was in the chart or the electronic medical records (where applicable). If nothing was noted in the chart, check the "Not Known" box. If the "Other reason" box is checked, write in what the reason was. This pertains to a reason that was listed in the records but is not captured by the reasons listed on the form. This is not data entered, but is kept in the source documents in the participant's folder at the FC.

End of Form

8.8.3 Liver Biopsy Pathology Report Summary Form

INSTRUCTIONS

This form is to be completed by abstracting the data off of the liver biopsy pathology report that was obtained prior to the Central Reading of liver biopsies. Please retain the original pathology report in the participant's HEIRS folder along with this form. The Adjudication Subcommittee may request to see the pathology report at a later time. **If there are questions or difficulties filling out the form, the HEIRS staff should check with their FC Adjudication SubCommittee representative for clarification.**

This form will be data entered into the HEIRS website data entry system.

Staff will complete the header at the top of the page, to include:

- Participant id and acrostic
- Date that the form was filled out
- Completed by: assigned digitial code or initials

Please record exactly what was in the pathology report and complete the following :

- Patient/Hospital Id # (For clinic use only; is not data entered)
- Clinic or facility where the biopsy was read (For clinic use only; is not data entered)
- 1. Date of the liver biopsy
- 2. Diagnosis related to iron overload-please check all that apply: (none indicated, alcoholic liver disease, cirrhosis, hepatitis C, hepatitis B, hepatitis-other, non-alcoholic steatohepatitis, fatty liver, fibrosis, primary hemochromatosis, secondary hemochromatosis, hemochromatosis not specified, hemosiderosis (secondary iron overload), other iron overload-please specify and other liver disease-please specify.
- 3. Stainable Iron
- Record the amount and distribution of stainable iron: biliary epithelium (absent, present, not reported), Mallory bodies (absent, present, not reported), Kupffer cells/Macrophages (absent, present/not increased, increased, not reported), Hepatocytes-prose only (absent, present/increased, increased, not reported). There are two separate grading scales for recording the grading: a four point and a six point scale.
  4. Hepatic Iron Concentration if available:
- Please check Yes (Y) or No (N) if the hepatic iron concentration is available from the patholgy report
- If yes, please record the weight (ug/g dry or wet weight, umol/g dry or wet weight)
- Participant's age in years at the time of the biopsy

End of Form

8.8.4. HEIRS Central Reading Liver Biospy Form

INSTRUCTIONS

Staff will complete the header at the top of the page, to include:

- Participant id only

The rest of the form will be completed by the central reader and will be data entered by the London Health Sciences Centre data entry staff.

The sample should be sent directly from the HEIRS FC along with this form. FC staff should make arrangements for the sample to be picked up or sent to the HEIRS office for shipment to London Health Sciences Health Centre. The sample and form should be sent via Federal Express to:

Dr. S Chakrabarti
Department of Patholgy
London Health Sciences Centre, University Campus,
339 Windemere Road
London, Ontario, N6A 5A5
Canada
Phone 510-685-8500 X36360 Fax: 519-663-2930
Email: subrata.chakrabarti@lhsc.on.ca

Please email Dr. S. Chakrabarti (**subrata.chakrabarti@lhsc.on.ca)** with the Fedex Tracking number at the time of shipment.

8.9. Protocol for Central Reading of Liver Biopsies

Beginning in June, 2003, the HEIRS study will perform central reading of all liver biopsies. Funding was approved in February 2003.

Central Reading Liver Biopsy Protocol

A) Preamble
It is expected that the referring center will obtain consent for these studies. The material should be sent using HEIRS participant id numbers only and without any name or clinical information attached. All material will be destroyed either during the quantitative iron analysis or at the end of the HEIRS Study period.

B) Preparation of material
The paraffin block or portion of the paraffin block of the liver biopsy is to be sent to London Health Sciences Centre. If it is not possible to send the block or portion, send:
1) 6 (5um) unstained sections on 6 positively charged slides; and
2) Multiple 10 um sections of paraffin embedded tissues collected in Eppendorf tubes with a total amount of liver tissue not less than 0.5 mg for quantitative iron analyses (if necessary).
Any available sample is preferable to none at all.

C) Shipping
Each Field Center is advised to comply with any local shipping requirements. London Health Sciences Centre has no special requirements for receipt of this material.

Materials are to be sent by Fedex to:
Dr. S. Chakrabarti,
Department of Pathology,
London Health Sciences Centre, University Campus,
339 Windermere Road,
London, Ontario, N6A 5A5.
Canada
Ph: (519)685-8500, X36350 Fax: (519)663-2930
(subrata.chakrabarti@lhsc.on.ca)

Please email Dr. S. Chakrabarti (**subrata.chakrabarti@lhsc.on.ca)** with the Fedex Tracking # at the time of shipment.

D) Tissue preparation and pathological assessment:
From the paraffin embedded tissue blocks approximately 6 sections will be cut.They will be stained with 1) Hematoxylin & eosin X2 2) Trichrome stain 3) Prussian blue (iron) 4) PAS- Diastase. If only unstained slides are received, only staining will be done, but not quantitation of iron. The tissue sections will be subjected to microscopic examination for pathological processes.

The report will consist of specific information with respect to:

a) Presence of stainable iron

i) Yes or No

ii) If yes: - in which cells? a) macrophages, b) hepatocytes, c) biliary epithelium

iii) Iron quantification will be carried out using the method described by Scheuer et al* .Scoring of stainable iron in the hepatocytes will be performed and a numeric score from 0-4 will be assigned to each biopsy.

b) Presence of fibrosis and or cirrhosis

The biopsies will be assessed for fibrosis and will be assigned a score of fibrosis ranging from 0 (no fibrosis) to 4 (cirrhosis).**

c) Presence of other abnormalities such as chronic inflammation, alpha-1 anti-trypsin granules, if detected during the examination will also be reported.
All tissues will be subjected to iron quantification by atomic absorption spectrophotometry.

Results will be reported using the HEIRS Central Reading Liver Biopsy Form.

*Scheuer et. al. J. Pathol. Bacteriol 84:53-64, 1962.
** Desmet et al. Hepatology 19:1513-20, 1994.

E) Turnaround time:
The report of histological analyses will be sent to the submitting Field Center within two to three weeks. Additional time is needed for iron quantification.

8.9.5 Annual Follow-up of Comprehensive Clinical Exam Participants

All participants **(except controls)** who have attended a CCE will be followed one year after their CCE. The data will be collected on the Annual Clinical Follow-up Forms (see below).

The purpose of these forms is to capture data about diagnostic tests, treatment, and response to treatment following completion of the CCE. **The form has been revised in July 2003, and now includes two separate forms:** One form (For Completion by Participants) is to be sent to and completed by the participant; the second form is to be sent to and completed by the participants' primary care physician or treating physician (For Completion by Clinician/Clinic Staff). It must be noted that at Kaiser sites, HEIRS staff may elect to perform electronic medical records abstraction of the data for the Physician Version of the Annual Clinical Follow-up Form.

HEIRS Investigators will be available to assist and to answer questions. In some cases, the form may be completed by one of the Investigators in consultation with the Primary Care Provider. Once the forms have been completed, the results will be incorporated into each patient's HEIRS database for analysis of outcomes

8.9.6 Procedures for Sending the Annual Clinical Follow-up Forms

The two forms that are used for annual follow-up of CCE participants are the Participant and Clinician versions of the Annual Clinical Follow-up Forms. Prior to sending out these forms, each Field Center should print off the applicable cover letter for the form from their local data base. There are two different cover letters; one for participants and one for clinicians/physicians. FCs should pay close attention to having the correct cover letter for each appplicable form. The Participant Contact Information Form or Family Contact Information Form should also be printed from the local data base. This will be used for the participant version only, and will be used to update any contact information that may have changed since the form was completed at Initial Screening. A summary of positive responses (items that the participant indicated "yes" to) from the Medical History Form at the CCE should also be printed from the web site reports and be included in both participant and physician/clinician versions of the forms. This summary of positive responses is available on the HEIRS web site under the "Tracking" menu, then "Print Medical History Summary", where you enter the participant's id and hit the "Go" button and the summary will print (see below).

1 Year Followup

Print Medical History Summary: GO

After the above steps are taken, the procedures include the following:

The Annual Clinical Follow-up Form, For Completion by Participants is sent directly to the participant. This mailing includes:

1. the participant cover letter,
2. the Annual Clinical Follow-up Form-Participant Version,
3. the summary of positive responses from the Medical History Form at the CCE,
4. the Participant Contact Information Sheet (or Family Contact Information Sheet for Family Study members),
5. and a self addressed, stamped envelope for easy return of the completed form.

This form is sent FIRST, before the Physician version. The reason for this is so that if the participant has changed primary care physicians, or is seeing another physician for treatment of iron overload, the FC will have this information available from the item # 17 of the Participant version. FCs should check with their Principal Investigator to determine if a HIPAA authorization should be included with the form. Some FCs may require this to be signed by the participant prior to sending the form completed by clinician/clinic staff.

The Annual Clinical Follow-up Form, For Completion by Clinician/Clinic Staff is sent directly to the participants' primary care physician or treating physician (see information below). This mailing includes:

1. the clinician/clinc staff cover letter,
2. the Annual Clinical Follow-up Form-Physician Version,
3. the summary of positive responses from the Medical History Form at the CCE
4. and a self addressed stamped envelope for easy return of the completed form.

FCs are to check with their Principal Investigator (PI) to determine if a HIPAA authorization or a copy of the consent form should be sent along with this form to the primary care or treating physician.

Whom to send the Physician Version Form to?

- The first choice is if the participant is being seen or treated by an HEIRS physician, to send to this physician. This will vary by Field Center.
- If the participant is being seen by an invesitgator outside of the HEIRS study, but who collaborates with the Field Center for follow-up data, send to this physician.
- If the participant notes on the Annual Clinical Follow-up Form-Participant Version (item #17) that they have changed primary physicians or have been seeing another physician for treatment, and has indicated that it is okay for the HEIRS study to contact them, send to this physician. FCs should check with their PI to determine if

a HIPAA authorization or a copy of the consent form should be included with the form.

- If the above choices are not relevant, then check the Participant Contact Information Form for the name of the primary care physician, and check to see if the participant has indicated that it is okay for the HEIRS study to contact them; if this is yes, send to this physician.

FORMS

8.10 ANNUAL CLINICAL FOLLOW-UP FORMS - QUESTION BY QUESTION

8.10. 1 Annual Clinical Follow-up Form- For Completion by Participants

INSTRUCTIONS

Staff will complete the header at the top of the page before sending to the participant, to include:

- Participant id and acrostic
- The date of the participant's CCE.

The form is mailed to the participant (see section 8.9.6.above). FCs should check with their Principal Investigator to determine if a HIPAA authorization should be attached for question #17. Once the form is returned back to the FC, the form is checked for completeness. The staff person who reviews the form will then complete the following header information:

- Date that the form was filled out- This is the date that the form was returned to the FC and reviewed; and
- Completed by: assigned digitial code or initials-This is the person who reviewed the form for completeness.

This form includes seventeen items for the participant to answer:

1. Have you had any of the following: Additional evaluation for iron overload, phlebotomy or liver biopsy? The responses are yes or no.

Since the CCE, have you had any change in the following: (responses are improved, no change, worsened, or not applicable)

2. swelling of feet or ankles
3. change in skin color
4. unexplained weight loss
5. abdominal swelling or fluid
6. chronic fatigue/weakness

7. shortness of breath
8. joint stiffness/pain/ache
9. excessive thirst
10. polyuria (excessive urination)
11. unexplained abdominal pain or discomfort
12. unexplained confusion or memory loss
13. unusual bleeding (vomiting or coughing up blood, blood in stool, blood in urine)
14. abnormal heart rhythm, heart beat or action/arrhythmia
15. For Men Only: Trouble having an erection or loss of sexual drive
16. Have you experienced any other major health changes?
17. Have you changed primary care physicians, or are you seeing another doctor for treatment of iron overload? If yes, name and address of doctor.

Do you give permission for HEIRS to contact this doctor to obtain information about your health care as it relates to iron overload? Responses are yes or no. FCs should check with their Principal Investigator to determine if a HIPAA authorization should be attached for this question.

End of Form

8.10.2 Annual Clinical Follow-up Form-For Competion by Clinician/Clinic Staff

INSTRUCTIONS

Staff will complete the header at the top of the page, to include:

- Participant id and acrostic
- The date of the participant's CCE
-

The form is mailed to the participants' primary physician or treating physician (see section 8.9.6). FCs are to check with their Principal Investigator to determine if a HIPAA authorization or a copy of the consent form should be sent along with this form to the primary care or treating physician.

Once the form is returned back to the FC, the form is checked for completeness. The staff person who reviews the form will then complete the following header information:

- Date that the form was filled out- This is the date that the form was returned to the FC and reviewed; and
- Completed by: assigned digitial code or initials-Ths is the person who reviewed the form for completeness.

The form has twenty four questions and include:

1. Have you seen this patient since the date of the CCE (HEIRS staff fills in the date before sending). The responses are Yes or No.

2. What is the patient's current diagnosis? Check all that apply (hereditary hemochromatosis, iron overloading anemia, other iron overload, porphyria cutanea tarda, hepatitis B, C or other, no iron overload or other-please specify).
3. Has this patient died? If yes, date of death and cause of death.
4. Has the patient had any of the following (responses are yes or no): hepatocellular carcinoma or cholangiocarcinoma, liver failure, liver transplant; if yes, date of transplant.
5. Has the patient had any of the following (responses are yes or no): liver biopsy, quantitative phlebotomy, additional evaluation for iron overload.
6. Was the patient treated by erythrocytapheresis? This item is to determine whether red blood cells were removed but plasma was returned to the patient (erythrocytapheresis). Responses are yes or no. If Yes, indicate whether iron depletion was achieved.
7. Record the most recent results for serum ferritin concentration. Enter the date corresponding to the result. Enter the results of the patient's most recent transferrin saturation. Enter the date corresponding to the result.

8-23**.** These items are included to record the patient's response to treatment, if any. Please complete these items whether or not the patient has received treatment. If the patient initially had the manifestation listed, check to indicate whether it has improved, remained unchanged, or is worse. If the patient did not have a given manifestation initially, and does not have it now, check N/A and go on to the next item.

8. iron overload or hemochromatosis
9. anemia
10. sickle cell anemia
11. thalassemia or other inherited anemia
12. unusual blood loss
13. diabetes
14. liver disease
15. thyroid disease
16. heart failure
17. abnormal heart rhythm, heart beat or action/arrhythmia
18. other heart disease or heart attack
19. arthritis
20. osteoporosis
21. porphyria cutanea tarda (blistering skin rash made worse by sunlight)
22. HIV or AIDS
23. chronic inflammation, chronic infection, autoimmune disease or lupus

24. Has the pateint had chemotherapy or a bone marrow transplant since the HEIRS CCE? Responses are yes or no.

End of Form

8.11. Forms Review and Data Entry

The quantitative phlebotomy and/or liver biopsy data will be ascertained by the HEIRS staff for recording on the appropriate forms. It is important to go through the records in a timely fashion for gathering and recording the data. The HEIRS staff will also attempt to retrieve any missing data using participant medical records, and abstract the weekly treatment data for use in filling out the Treatment Session Form, and data entry into the central study database. It is up to each FC to develop routine methods of reviewing the participant records for data abstraction. Abstraction from electronic medical records will be performed where available.

The HEIRS data entry staff at each field center will be responsible for data entering the Treatment Session Form and the Monthly Treatment Summary Form into the HEIRS central study database. The first of these forms that is data entered is the Monthly Summary Form. It must be noted that if on the Monthly Summary Form, it is recorded that qph is NOT being performed monthly, then access to the Treatment Session Form will be denied. Additionally, HEIRS field center staff are responsible for data entering the Liver Biopsy Pathology Report Summary and the Annual Clinical Follow-up Forms-both Participant and Clinician Versions. Field Centers should always review the forms for completeness. Kaiser sites may elect to perform electronic medical records abstraction for filling out the Annual Clinical Follow-up Form-Clinician Version only. The Central Reading Liver Biopsy Form is data entered by the staff at London Health Sciences Centre, and the hard copy form is then sent back to the originating FC for filing in the participant's HEIRS chart.

8.12 Quantitative Phlebotomy Quality Control

Quality control for the HEIRS Study Quantitative Phlebotomy protocol will be performed in two ways:

1) The Central Laboratory will send a set of weights to each Field Center Study Coordinator who will facilitate the weighing process at each site used by their Field Center. A 50 gm and 500 gm certified standard weight will be evaluated on balances used to weigh the bags/bottles of blood removed during the HEIRS Study Quantitative Phlebotomy protocol.

2) Two pooled samples of EDTA whole blood will be prepared by the Central Laboratory and shipped to each Field Center for evaluation of hemoglobin and/or hematocrit using the procedure that will be used during the quantitative phlebotomy procedure. The pools will be shipped by overnight courier to each site where they will be refrigerated until analysis. Analysis will be performed within 3 days of receipt.

Field Center or phlebotomy site personnel will enter the results on the forms sent with the weights and samples and fax them to the Central Laboratory. A final summary report for each QC procedure will be compiled by the Central Laboratory and submitted to the Coordinating Center.

8.13 QUANTITATIVE PHLEBOTOMY QUALITY CONTROL FORM

Purpose: Quality control for the HEIRS Study Quantitative Phlebotomy protocol will be performed in two ways: 1) Standard weight will be sent to each site to be checked on balances used to weigh the bags/bottles of blood removed during the HEIRS Study Quantitative Phlebotomy protocol and 2) A pooled sample for hemoglobin and/or hematocrit determination will be sent to each site for analysis.

Balance Check Procedure:

1. Record the manufacturer, model, and serial number of the balance on the table below.
2. Weigh each of the standard weights in duplicate on the balance used for weighing the blood bags/bottles from HEIRS participants as part of the Quantitative Phlebotomy protocol.
3. Record the weights on the table below. (If more than one balance is used, record the information for each on a separate form.)

Balance Manufacturer:

Model: **Serial Number**:

	50 g weight	500 g weight
1st weighing		
2nd weighing		

Hemoglobin/Hematocrit Procedure:

1. Within 3 days of receipt of two pooled samples, analyze each sample for hemoglobin and/or hematocrit using the method that will be used in the HEIRS Quantitative Phlebotomy protocol. Keep samples refrigerated until analysis is performed.
2. Record the method and instrumentation information and the sample results on the table below. If either hemoglobin or hematocrit is not performed, record "ND" for the result. (If more than one instrument is used, record the information for each on a separate form.)
3. If hematocrit was performed, record the presence or absence of hemolysis by circling the word that best describes your observation.

Method: **Instrument Manufacturer:**		
Model:	**Serial Number:**	
	Sample #1	Sample #2
Hemoglobin (mg/dL)		
Hematocrit (%)		
Presence of hemolysis (circle one)	None / Slight / Medium / Gross	None / Slight / Medium / Gross

Fax completed forms to Cathie Leiendecker Foster at 612-273-6994

9. RETENTION

For the HEIRS Study, retention includes facilitating completion of the screening visit, recruiting eligible screening participants into the second phase of the study (the CCE) and the ELSI focused follow-up phase, and recruiting eligible CCE participants into the family study.

9.1 Factors Affecting Retention

1. The salience (perceived importance) of the research topic to the participant is probably the strongest single factor in retention. The salience of this research will be greatest for those with a personal interest in iron overload, whether because they have been diagnosed phenotypically, or screened positive for a mutation, or are immediate family members of these groups. For these groups, the repeated message of the treatability of iron overload, if detected early and the promising role of gene testing in detecting those at risk, and the need for better natural history information for some subgroups, should be delivered to enhance retention. Topics to discuss on the phone call to CCE-eligible participants are in Appendix D.
2. For controls (defined as not being in a known or suspected phenotypic or genotypic subgroup), the salience will be much lower and strategies to minimize censored observations are therefore limited to basic sound research practices. Topics to discuss on the phone call to potential controls are in Appendix D.
    a. Accurate records, containing names and contact information for non-participants who will likely know the whereabouts of lost participants (Participant Contact Form), is required for successful tracking, either to re-enroll the participant or to determine the reason for loss of contact.
    b. Participant burden should be minimized by reducing duplicated data requests to those required for error checking or other essential data management tasks.
    c. Participants should have a phone number to use if they have questions or concerns about the study, and should have access to senior local project staff if questions or particularly concerns or complaints are unresolved.
    d. To aid in establishing and maintaining rapport, where possible, participants should be assigned the same interviewer and study contact person over the course of repeated contacts.
    e. Participants should be kept informed of study progress using regular mailings or newsletters, etc. (Newsletter for all CCE eligible participants and controls is being developed)
    f. An open public forum can be utilized for all CCE eligible participants to discuss the HEIRS study at each field center.

9.2 Maximizing Continued Participation by HEIRS Study Participants

The study will be most efficient if we can maintain the participation of individuals who have agreed to participate and who have already completed part of the study

requirements. But we have to accept the fact that not every participant will choose to continue participation once she/he has begun, and respect each participant's decision whether or not to continue.

Some decisions about continuing participation may be due to barriers that the HEIRS Study can help overcome (e.g., providing financial assistance for child care so that a participant can attend the CCE). It is important to speak with participants about their decisions not to continue, if they are willing to speak with us.

Procedures for maximizing continued participation include:

- Sending reminders for scheduled appointments
- Contacting a participant right away about a missed appointment or missing information, to understand why the participant did not do what was expected and to resolve any outstanding concerns or issues
- Offering assistance when appropriate (e.g., financial assistance with transportation or child/elder care)
- Acknowledging participants' contributions to the HEIRS Study
- Offering incentives for continued participation (this may require IRB approval so check with local IRB)
- Approaching problems and participant concerns in a non-judgmental way, always looking for solutions that will meet participant and study needs

Incentives for continued participation in the CCE or family study may include cash/gift certificates, small items (e.g., study mugs or tote bags), study updates, and/or holiday/birthday cards. Regular mailed contacts will also help us keep track of current addresses for participants.

9.3 Managing Participant Losses

Participants will not continue in or be lost to the study for many reasons:

- Death or illness
- Death or illness of a family member
- Moving out of the area
- Logistical problems (e.g., busy work or personal schedule; family responsibilities)
- Dissatisfaction with the study
- Lack of interest

Some of these losses are unavoidable. It's our job to try to improve the HEIRS Study so that the participants will want to and be able to continue participation. When possible, it's important to talk with participants about leaving the study and offer solutions that may meet the needs of the participants and the study. It's also important to present problems and discuss possible solutions with senior HEIRS Study staff, as designated at each Field Center.

Possible solutions include providing reimbursements for transportation and child/elder care, and offering alternative visit schedules.

Some participants may be more likely to continue participation if a study doctor contacts them. Consider having a study doctor call reluctant participants, or members of cultural groups in which this contact is likely to be more effective than staff calls.

Alternative visit schedules may include:

- Offering to delay the visit. Participants who cannot come in for a visit now but may be willing to come back at a later date (e.g., 3 months later) may be called back to schedule a later, more convenient visit.
- Offering a split visit (i.e., completing the visit in more than one session). Ideally, all parts of the CCE will be completed within a week. A split visit may be particularly useful for participants who cannot attend a full visit on a weekday, but are willing to provide a fasting blood specimen during the week and complete the visit on the weekend.
- Offering a visit in a place other than the clinic. Consider conducting the CCE in the personal physician's office, by either HEIRS Study staff or the participant's personal physician, at an alternate clinic site by HEIRS Study staff, or in the participant's home. The two primary concerns are: 1) whether blood specimens can be processed and shipped according to HEIRS laboratory procedures if the CCE is performed in an alternative location; and 2) training for examiners not associated with the HEIRS Study. If the examiner is not associated with the HEIRS Study, consider sending an HEIRS Study staff member (e.g., research nurse) to assist the examiner.
- Offering a weekend visit. The primary concern is whether the blood specimens can be picked up and delivered reliably over the weekend. Do not ship on the day before a holiday or on Saturdays without first checking with the Central Laboratory. Also, check with Federal Express about pick-up schedules on weekends. There may be limited service available in some areas.
- Coordinating the CCE or blood draw with a regular clinical visit. The two primary concerns are: 1) whether blood specimens can be processed and shipped according to HEIRS laboratory procedures if the CCE is performed in an alternative location; and 2) training for examiners not associated with the HEIRS Study. If the examiner is not associated with the HEIRS Study, consider sending an HEIRS Study staff member (e.g., research nurse) to assist the examiner.
- Collecting non-fasting blood samples if it is not possible to obtain a fasting sample. If a participant presents for his/her CCE and is not fasting, it is better to collect a non-fasting blood sample than to collect none at all.
- Offering a reduced visit. If the participant is unable to attend a full visit, then offering a visit where only part of the visit measurements are obtained may provide the minimal necessary information. Consult senior HEIRS Study staff, as designated at each Field Center, to determine the priority list for the CCE components.

9.4 Defining Losses to Follow-up and Non-participants

An individual may be lost to follow-up because she/he has died, moved away from the area, or can't be found. She/he may be hard to reach, may decline continued participation, or may continue to say "Yes" and not follow-through. This section contains guidelines for contacts and defining losses to follow-up or non-participants. These guidelines apply to potential cases and controls, as well as the initial recruitment of family members for the family study.

In the situations below, a participant may be considered a non-participant for the CCE.

1. Declines to continue in the study
2. Death
3. Moved out of the area; new residence not in a location close to another HEIRS Study Field Center
4. Cannot locate after using all available contact information, including secondary contacts provided at the screening visit, and other tracking methods approved by your IRB

Below are minimum criteria for attempted visits and contacts. If one of these is met, a participant may be considered a non-participant for the CCE. A Field Center may decide to extend contact attempts for certain individuals or for all of their participants.

1. 3 scheduled CCE visits in which the participant does not attend (DNA or "no-show").
2. 3 contacts with the participant without scheduling a CCE appointment.
3. More than 6 months have passed since screening results sent, and a CCE visit has not been scheduled.
4. 10 calls have been made with no contact with the participant. Attempts include at least 2 daytime, 2 evening, and 2 weekend calls over a 2-month period. The participant's preferred calling days/times should be considered. It is not necessary to leave a message every time; some participants may find this annoying or consider it harassment. Leave at least 2 messages over the 2-month period.

It may be helpful to send a letter to participants who are hard to reach, providing one more opportunity for them to participate in the CCE.

9.5 Role of Clinic and Research Staff Relationships in Retention

As with initial recruitment, participants may judge the study and us by our relationships with clinic staff as well as with them. Participants may ask their clinicians or clinic staff about staying in the study. We need to continuously monitor our relationships with clinic staff and clinicians, so that we will be able to solve any problems as quickly as possible. This may include daily "check-in" with clinic managers or leaders, or regular staff meetings. Clinic staff may also appreciate periodic study updates and thank-you gifts (e.g., cookies or candy). Maintaining research staff morale and interest also requires some attention. Project staff may have a better sense of cohesiveness and support by

having regular staff meetings, focusing on accomplishments and what's working well in addition to problem-solving difficult issues or situations. Morale and cohesiveness may also be increased by having periodic celebratory events, and by providing small study-related items as gifts.

10. Quality Assurance / Control

10.1 Overview

Quality assurance and control are the responsibility of every member of the HEIRS team. This section of the MOP will describe the quality assurance and control philosophy and procedures to be used in HEIRS.

Quality Assurance activities are those performed before the data are collected, to minimize the number of data errors that occur. Quality Assurance activities in HEIRS will include: (1) a well-documented, standard protocol to be performed at all sites in an identical manner; (2) centralized training of key clinic staff so that all clinics perform HEIRS procedures in the same way; (3) requirements regarding demonstrated proficiency in administering questionnaires/interviews and minimum number of procedures required to obtain and maintain certification; (4) routine observation of clinic staff to verify adherence to protocol; (5) routine calibration of equipment used during the screening and comprehensive examinations; and (6) clinic site visits to verify overall adherence to established protocols.

10.2 Centralized Training of Key Staff

Primary steps in assuring good quality of study data are adequate training and periodic observation of study personnel. A highly motivated, conscientious staff may be the best guarantee of data quality. Other key considerations include adequate monitoring of technician performance by supervisory staff at the Field Centers and support units. Such monitoring can identify and correct problems weeks or months before they would become apparent from Quality Control activities such as statistical analyses performed by the CoC.

An approach toward training that should be most efficient for HEIRS is the "train the trainer" model. In this model, each FC appoints a Training and Quality Assurance liaison who assumes overall responsibility for training (and re-training) of other clinic staff and verifies that all staff adhere to established study protocols. In general, training on specific components of the HEIRS protocol will consist of a detailed process review, an item-by-item review of the relevant forms(s) including rationale for inclusion and instructions on proper form completion, demonstration of techniques where applicable, and practice sessions. Training of other staff will be conducted by FC Training and QUALITY ASSURANCE (QA) liaisons following protocols, guidelines and standards established during Centralized training. The training materials developed for the initial training session will be made available to the field centers and disseminated as needed to new staff and existing staff responsible for their training. The training and re-training

requirements for study components are summarized below. Specific instructions are described in detail in the relevant chapters.

10.3 Certification and Recertification

Field Center Training and Quality Assurance (QA) liaisons

Certification of Field Center Training and Quality Assurance (QA) liaisons will occur during centralized training for physical examination components of the comprehensive examination based on certification standards established by the Screening and Exam Committee. Re-certification of Field Center Training and Quality Assurance (QA) liaisons and other staff will occur periodically as specified by the HEIRS Study Protocol or as necessary as evidenced by QC results.

Non-Field Center Training and Quality Assurance (QA) staff

Certification of Non-Field Center Training and Quality Assurance (QA) staff will be conducted on-site by Field Center Training and Quality Assurance (QA) liaisons following the protocol and standards established in the Centralized Training session.

10.4 Quality Control

Quality Control activities are those performed during or after data are collected, to identify any errors which have occurred. Quality control in a large study such as HEIRS has two major purposes: (1) to identify problems in data collection and measurement in time to institute appropriate corrections; and (2) to quantify the quality of data collected over the course of the study so as to provide information necessary to interpret study results. To accomplish the first goal, adequate data must be accumulated to enable valid analyses to be performed within a brief period after initiation of data collection. To accomplish the second goal, sufficient data must be compiled throughout the study to detect any drift or deterioration in data quality over time. Because of finite resources, both in staff and in acceptable burden on participants, each component of a quality control program must be selected on the basis of assessing the need, feasibility, and overall importance to the main goals of HEIRS.

Data from the Central and local laboratories are among the most important collected by HEIRS. High quality data must be obtained from these units in order to fulfill the primary goals of the study. For these reasons, the Laboratory/Quality Assurance Committee will place special emphasis on quality control of these units.

For the other study components (e.g. data management), the CoC can provide considerable quality control information by relatively simple analyses of data acquired from all sites. Monitoring of the distribution of individual values and of mean or median values by center and clinic may identify many problems. Because of the large numbers available, this will be a particularly useful way of detecting many problems. For more specific information on data management, please refer to Chapter 11, Data Management.

10.4.1 Data Entry Quality Control

Special quality control aspects of the data entry process are described in detail in Chapter 11, specifically, 11.2.6 - Screen Data Validation Procedures and 11.2.7 - Guidelines for Data Entry. Approaches to assuring the quality of data entry will include double data entry, range and logic checks, and the generation of data edit queries and data quality reports. During the Initial Screening recruitment, the CoC will perform periodic data entry quality control checks of approximately 2% of participants until the FCs maintain a consistent acceptable error rate. The study goal is an error rate of less than one half of one percent. A randomized list of participant ids are sent to each FC, with instructions to photo copy the Initial Screening Form for each of the ids and send to the CoC. The CoC will then perform double data entry checks on these forms. The error rates for each FC are tabulated and posted on the HEIRS website, under the Data Entry Reports of the Quality Control Reports section.

The same approach will be utilized during the Comprehensive Clinical Exam phase and the Family Study phase of the study.

10.4.2 Data Queries

The CoC generates weekly Initial Screening data queries for all of the FCs and the Central Laboratory. These weekly data queries are posted on the HEIRS web site, under the "Reports" menu, then "Quality Control", and are field center specific listed as Data Queries. Each FC will only see their queries. The CoC sends the FCs an email each week, notifying the FC that the week's data queries are posted on the website. FCs then can look at the queries and address the questions weekly. The queries list the HEIRS id, the date of the IS visit, the specific form with the query, and whatever the query may be. Examples of these queries include missing items on the Medical History Form that are necessary for classification of iron overload, Initial Screening Form that has not been data entered, the Fairview id is missing or the consent date is greater than 11 months from the date of data entry.

The same procedures for data queries will be initiated for the CCE and the Family Study phase of the study.

10.5 Laboratory Quality Control

Quality Control Duplicate Blood Samples (Replicate Samples)

As part of the overall quality control program for laboratory analyses, duplicate specimens are sent to the laboratory, with one half of each specimen pair sent under the participant's regular HEIRS Laboratory ID number, and the other half under a HEIRS Quality Control Phantom Participant (QC) Laboratory ID number. The HEIRS QC Laboratory ID numbers are not distinguishable from other HEIRS Laboratory ID numbers so that this forms a blinded external quality control program monitoring measurement variability.

To create the phantom duplicates, a second set of tubes is collected from each approximate 50th Initial Screen participant (2 percent) and every 20th CCE participant at each site and sent out under HEIRS QC Laboratory ID numbers. **Only blood collection tubes #1, #2, #3 and #4 will be collected for QC samples at the CCE.** (Please see Chapter 13, section 6.2 for more specifics on CCE) Results on each laboratory measurement are matched to the appropriate participant results at the CoC.

Weekly Blood QC Sample Checklist

The HEIRS FC laboratory technicians maintain a weekly checklist of the QC samples to be collected during the week. As soon as each sample is drawn and processed, the corresponding labels are placed on the chart, and it is checked off. An example of the checklist is given below. The finished checklists should be kept in a permanent file at the field center.

Weekly Blood QC Sample Checklist

Week of: _____

QC Event #	HEIRS Laboratory ID	HEIRS QC Laboratory ID	Collected?
1	_____	_____	_____
2	_____	_____	_____
3	_____	_____	_____
4	_____	_____	_____

Collecting and Processing QC Samples

Every 50th HEIRS Initial Screen Laboratory ID label and every 20th CCE Laboratory ID label is highlighted with a colored marker to remind the technician that QC samples need to be collected. (The participant who is assigned the highlighted labels does not necessarily have to be the volunteer for the QC samples.) When a participant is selected for the QC sample collection, select a HEIRS Laboratory ID number that is NOT sequential with the HEIRS Laboratory ID number that is being assigned to the participant.

Selecting Participants for QC Blood Draw: Collect QC specimens from participants who have quality veins, and from whom blood is easily collected.

A NEW NEEDLE STICK SHOULD NOT BE DONE JUST TO GET MORE BLOOD FOR A QC SPECIMEN

Order of QC Tubes in Relation to Regular Blood Collection: Collect the QC tube(s) after the other two tubes have been filled (for IS) and after the six tubes have been filled for the CCE.

To reduce the burden upon HEIRS CCE participants, no one person is asked to contribute sufficient extra blood to make a complete set of duplicates for all tests. Instead, extra blood is drawn from two participants and sent to the Central Laboratory under 2 different "phantom" HEIRS ID numbers and QC LAB ID numbers. **Only blood collection tubes #1, #2, #3 and #4 will be collected for QC samples at the CCE.** The first volunteer participant will donate additional red/gray stoppered tubes #1 and #2. The second volunteer participant will donate additional lavender stoppered tubes #3 and #4. When a participant is selected for the QC sample collection, select a new Lab ID number and a phantom HEIRS ID# (from the screening ID# list as is done for the screening QC samples) for these replicate samples. For the first participant who volunteers to donate a QC sample (tubes #1 and #2), place a Lab ID# label on the Clinical Assessment form 6, page 2 in the box next to "Fairview replicate" and a second label with the same Lab ID# on the Lab Test Request Form used for the first QC donor. Also, place a phantom HEIRS ID# on this Lab Test Request Form and enter "HEIRSX" for the acrostic. For the second participant who volunteers to donate a QC sample (tubes #3 and #4), place a different Lab ID# label on their Clinical Assessment form 6, page2 in the box next to "Fairview replicate" and a second label with the same Lab ID # on a Lab Test Request Form used for the second QC donor. Also, place a different phantom HEIRS ID# on this Lab Test Request Form and enter "HEIRSX" for the acrostic

Processing QC Blood: QC blood samples are processed along with the regular blood samples.

10.6 Quality Control Reporting

Numerous website reports are generated to communicate information about quality control. As the study progresses and more data becomes available, the CoC will generate new reports, graphs and charts to be placed on the HEIRS website. Additional data analysis and reports will be created in response to abstract/manuscript publications and presentations requests. The HEIRS website will be updated as the study progresses and data reports become available

Currently, (as of January 2003) the HEIRS website incorporates the following quality control reports (under the "Reports" menu, then "Quality Control"):

Data Queries
- Alabama
- California
- Howard
- Kaiser - Portland
- Kaiser - Hawaii
- Canada - London
- Canada - Toronto
- Fairview Edits

Data Entry Reports

Initial Screening Missing Values

Data Entry Forms Entered

Missing Fields Report

QC Replicate Report

Potential Duplicate Participant List

Additional quality control reports will be generated as the study progresses and the needs are identified.

10.7 Site Visits

During recruitment and follow-up, a site-visit team will site visit each field center to promote communication, answer questions, and ensure that study procedures are understood and carried out correctly. Site visits to the Coordinating Center (CoC) and the Central Laboratory may also be scheduled. Quality Control Monitoring at the site visits should involve the following:

1. assessment of recruitment (if appropriate according to study schedule including strategies and yield)

2. confirm presence of regulatory documents (IRB approval and annual renewal)

3. evaluate protocol adherence including verification of consents, inclusion criteria, and source documentation for at least 5 participant charts per year

4. assure that the participants visits are being conducted according to protocol guidelines

5. confirm presence of lab forms, QC checklists, shipping logs, etc.

6. confirm adequate study supplies (including Manual of Operations, protocol, forms)

7. trouble-shoot problems (e.g. retraining of new personnel, editing on-site corrections)

8. aid in correcting questions or edits for site to correct

9. follow-up report to the site, CoC, and Project Office;

10. confirmation from the site that requested follow-up procedures were completed.

10.7.1 Field Center Site Visits

For the field centers, the site visit program will provide a mechanism to encourage the effective and standardized delivery of recruitment efforts, intervention programs, and the collection of appropriate and valid data within each of the HEIRS field centers. Site visits may also be performed if consistent departures from the Protocol and Manual of Procedures are detected. Retraining and/or recertification may be done as needed during these visits, depending on availability of staff.

Prior to the site visit, the field centers and the CoC will generate a proposed agenda and a schedule will be worked out in advance. This will enable us to examine recruitment efforts, adherence and standardization of clinical procedures, as well as any staffing problems clinics may be experiencing.

The site visit will be an ideal time for suggesting solutions for any problems which are identified. It should be noted that outside visitors may not have better answers, however they may have different answers that may prove useful. Of equal importance will be the lessons that site visitors gain while watching other centers in action. The observational experience can enhance and increase the visitor's own skills at developing problem solving strategies and solutions that may be applicable to other clinical centers. Consequently, the site visits will be a time when the CoC staff, peers and clinic site staff review progress and problems, share what has/has not worked, and consider new strategies and solutions.

A key to a successful site visit is adequate preparation by the clinical center, the CoC, and the site visit team members. Site visits should serve to enhance communication throughout the study, and to personalize interchange among study staff and investigators. The site visit team members will consist of the Project Office, the CoC, a PI and a clinic coordinator from a different field center.

To make the site visit process as efficient as possible, the field center and the site visit team members will receive the Field Center Site Visit Checklist (Section 10.8) prior to the site visit. This will serve as a general tool to use during the site visit to ensure the success of the site visit, as well as give information concerning what to look at. This

checklist also aides the field center being visited in preparing for the site visit. Examples of questions for the site visit include the following:

1. Do clinics have an adequate number of staff members to provide for effective recruitment, data collection, other procedures, and data entry? Do staffing patterns match those originally proposed?

2. Are staff roles clearly defined and is there communication and interaction between the various working groups?

3. How is information, such as changes in the MOP or protocol, shared among personnel?

4. What is the overall view of clinic flow?

5. A clinic tracking system may be discussed and the following questions may be asked:

    a) What is the procedure followed when a participant does not show up for his/her appointment?

    b) How does the clinic handle rescheduling for a situation where the participant only partially completes a visit as a result of illness, etc.?

    c) How does the clinic keep track of where an individual is in the study flow?

Other activities that will take place during the site visit may include discussions of data reports provided by the CoC and exploration of any concerns or questions that arise. During the site visit, the site visit team may ask to follow a participant through a CCE visit and observe randomly selected procedures; as well as observe the recruiting efforts made in the clinics.

Chart reviews will also be a major component of the site visit. Questions will be asked about where records are kept and how participant confidentiality is assured. A site visitor will review at least five randomly selected chart reviews to look at items such as: informed consent, appropriate signatures, and complete data forms. Site visitors will ask questions concerning study documentation. Some typical questions will include (also included in the Site Visit Checklist):

1. Where is the MOP located in the clinic and do clinic staff have easy access to it?

2. Do the protocol and MOP have all the updates included?

3. What procedures are used to ensure that changes to the protocol or MOP are implemented in every day clinic operations?

4. Where is the staffing documentation, including certification and recertification, kept?

5. If applicable, what is the procedure for maintenance on equipment?

6. Where are IRB documents kept and what is the procedure for informing the IRB of protocol changes?

After each site visit, a site visit report will be generated by the CoC, in conjunction with the entire site visit team. There will be a frank discussion at the end of the visit between the site visit team, the principal investigator and key staff at the field center. The site visit team will prepare a written report on the activities of the site visit. A detailed report of the team's observations and recommendations subsequently will be sent to the Principal Investigator of the site being reviewed and Project Office. The field center that was site visited should respond to the report's recommendations, in writing, and copies go to the CoC and the Project Office.

The Central Lab and the Coordinating Center may be site visited during the study.

10.8 HEIRS Field Center Site Visit Checklist

The site visit is performed to promote communication, answer questions, and ensure that the study procedures are understood and carried out correctly. Items to observe during site visit include the following:

STUDY DOCUMENTS

Manual of Procedures (MOP)

Where is the MOP located?
Does the clinic staff have easy access to it?
Are the updates/revisions current?
How is the information, such as changes or updates, shared among personnel?
What procedures are used to ensure the changes are implemented in every day clinic operations?

Regulatory Documents

Where is the IRB correspondence kept?
What is the procedure for informing IRB of protocol changes?
What is the procedure for informing the project office and IRB of adverse events and protocol violations?
Are the IRB documents up-to-date?

Participant Charts (need to review at least 5 charts selected by the site visit team)

Was informed consent obtained?
Were appropriate signatures obtained?
Are the source documents included and are they complete?
Is there documentation for lab work (where applicable)?

Are the charts in a secure location for security and confidentiality?
How is participant confidentiality assured?
Are participant visits being conducted according to protocol guidelines?
Have the appropriate versions of the forms been used?
Are the data corrections documented correctly?

RECRUITMENT

What is the procedure for recruitment?
Are the Daily Recruitment logs accurate and up-to-date?
Are the Weekly Recruitment Summary Forms accurate and up-to-date?
Are the Participant Follow-up Logs accurate and up-to-date?
Are recruitment goals being met?
Are forms reviewed for completeness and accuracy with participants?
Are there any recruitment problems or issues?

LAB

What are the procedures for blood collection?
How are the samples processed?
How are the samples shipped and what is the timetable? (hazardous shipping)
Are the laboratory logs accurate?
Confirm presence of lab forms, QC checklists, shipping logs, etc
What is the lag time between available results and reporting of results?
Are OSHA/safety/universal precautions requirements met and adhered to?

STAFF

Where is the staffing documentation, including certification and recertification?
What is the procedure for new staff orientation and training?
Is there adequate staffing for effective recruitment, data collection and data entry?
Are there periodic staff meetings?
Are staff roles clearly defined and is there communication and interaction between the various working groups?

CLINIC

What is the overall flow of the clinic?
Clinic tracking discussions should include:
How does the clinic keep track of where an individual is in the study flow?
What is the procedure in handling “no-show” participants?
How does clinic handle rescheduling for situations where the participant can only partially complete a visit due to illness, etc.?

DATA MANAGEMENT/QUALITY CONTROL

What is the lag time for data entry for the different study forms?
How is the security of the computer and files handled?
Is the staff always using their own computer “accounts”?
Are computer backups performed and what is the frequency?

Who is responsible for resolving data queries? What is the lag?
Have charts selected for data entry quality control been sent to CoC?

FORMS INVENTORY

Where are the forms kept?
Are they in a secure location?
How is the supply of forms tracked to ensure there is always adequate supply?

ADDITIONAL ITEMS

Answer questions, trouble-shoot any detected problems
Is there routine calibration of equipment used?

Hereditary Hemochromatosis and
Iron Overload Screening (HEIRS) Study
Clinical Comprehensive Exam and Family Study Visit

Manual of Operations:
Central Laboratory
Specimen Collection and Processing

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TABLE OF CONTENTS

1. PURPOSE

The Hereditary Hemochromatosis and Iron Overload Screening Study (HEIRS) provides a framework for research into the genetic, biochemical, epidemiological, and physiological aspects of iron overload disease. The study participants for the Comprehensive Clinical Exam and Family Study will include approximately 4,000 individuals whose examination will include a battery of biochemical, hematological, and genetic tests.

There are five Field Centers involved in the study: Birmingham, AL (University of Alabama-Birmingham); Irvine, CA (University of California-Irvine); Washington, DC (Howard University); Portland, OR (Kaiser Permanente Center); London, ONT (London Health Sciences Centre). The technicians at the Field Centers collect blood specimens, process them, and ship them to the Central Laboratory at Fairview-University Medical Center in Minneapolis, MN. A complete list of tests performed is located in Appendix I.

The foundation upon which all of these tests are based is the blood specimens that are collected and processed by the technicians at the Field Centers. Probably the most important step (and potentially the most variable) is the collection and processing of the samples. Laboratory tests can be repeated, but if the sample itself is not correctly collected and processed, the laboratory results may be precise, but perhaps not reflective of the *in vivo* state. It is important that this study measures true differences between participants rather than differences in collection procedures. The HEIRS depends heavily on the Field Center technicians who perform the blood collection and processing. It is important that these people be not only well trained and competent at drawing blood and processing it, but also willing to take pride and responsibility in their work.

2. PREPARATION

2.1 Participant Contact

Since the study depends on the voluntary participation of participants, every effort must be made to make the entire procedure as easy and painless as possible for them. The technicians should wear a clean laboratory coat, remain calm and project an attitude of competence even when faced with the most nervous or inquiring participant. The best way to achieve this is for the technicians to be thoroughly knowledgeable about all aspects of the procedures.

2.2 Staff Certification Requirements

Certification of Field Center Training and Quality Assurance (QA) liaisons will occur during centralized training for the laboratory components of the comprehensive examination. Each Field Center appoints a Training and Quality Assurance liaison who assumes overall responsibility for training (and re-training) of other clinic staff and

verifies that all staff adhere to established study protocols. Training and certification of laboratory staff will be conducted by Field Center Training and QUALITY ASSURANCE (QA) liaisons following protocols, guidelines and standards established during Centralized training. Re-certification of Field Center Training and Quality Assurance (QA) liaisons and other staff will occur periodically as specified by the HEIRS Study Protocol or as necessary as evidenced by QC results. Appendices V and VI are example certification checklists and practical and written examinations that can be used for training and certification of laboratory staff.

2.3 Blood Collecting Trays and Tubes

Prior to venipuncture two trays are prepared for each participant. One tray holds the Vacutainer tubes used in the blood collection. The other tray holds the plastic microvials that contain the final serum and cell specimens that are sent to the Central Laboratory or local laboratory for analyses. The collection tubes and storage microvials are labeled with LABID numbers. A list of equipment, suppliers, and vendors is provided in Appendix II.

2.3.1 Blood Collection Tray

First, the technicians organize and prepare the blood collection tray. The tray itself should be made of hard plastic, which is unbreakable and can be easily cleaned. The tray has individual compartments, filled with the following supplies.

- A test tube rack to hold the three types of blood collection tubes drawn from each participant. These tubes are described in detail in the next section.
- Sterile, disposable 21 gauge needles
- Plastic Vacutainer tube guides
- Sterile alcohol swabs
- Gauze sponges
- Tourniquets
- Bandages ("Band Aids") or equivalent covering
- Smelling salts, ice packs, and wash cloths should be readily available in the specimen collection area for patients who become faint during the blood draw

2.3.2 Blood Collection Tubes

About 60 mL of blood is drawn from each participant using six Vacutainer tubes. Specimens from these blood collection tubes are used in several different assays. It is

important that the technicians are familiar with the purpose of each tube, the type of anticoagulant in each tube, and possible sources of error in the handling of each tube. The tubes are organized in the test rack in the following sequence:

Tubes #1 and 2 are 10-mL red and gray-stoppered tubes each filled with 9.5 mL of blood. This tube does not contain anticoagulant, so it does not need to be mixed following collection. After drawing, allow the blood to clot at room temperature for 30 minutes. Following centrifugation, the serum is frozen and sent to the Central Laboratory. If the serum is allowed to remain in contact with the red cells for much longer than 30 minutes, the risk of hemolysis increases. Significant hemolysis can effect the measurement of several of the tests including bilirubin, LDH, ALT, AST, GGT, and insulin.

Tubes #3 and 4 are 10-mL lavender-stoppered tubes containing the liquid anticoagulant EDTA. The plasma from tube #3 will be stored at the Central Laboratory for future testing and the packed cells will be frozen and shipped to the Central Laboratory for isolation of DNA, which is used for genetic analyses. Tube #4 will be used for CBC and reticulocyte count. After each of these tubes is fully filled with blood, gently invert 8 - 10 times; tube #3 is placed into a room temperature rack until centrifugation and tube #4 should be stored refrigerated until shipment.

Tubes #5 and 6 are 8-mL blue and black–stoppered tubes containing sodium citrate anticoagulant, gel material and Ficoll Hypaque solution. These tubes are sent to the Central Laboratory at ambient temperature and will be used for cryopreservation of lymphocytes for potential future immortalization. After each of these tubes is fully filled with blood, gently invert 8 - 10 times. (These tubes are provided by the Central Laboratory.)

2.4 Storage Microvials and Caps

The technician prepares a rack of the plastic microvials that will contain the final samples to be shipped to the Central Laboratory. The rack should comfortably hold tubes having a 10 mm diameter. A supply of red and yellow microvial caps should be available near the microvials.

2.4.1 Organization

The technician will need the following vials for each participant:

6 - 2.0-mL polypropylene microvials (red cap)
2 - 2.0-mL polypropylene microvials (yellow cap)
1 – 8.0-mL polypropylene vial (purple cap)

2.5 Preparation for Specimen Collection

Prepare for specimen collection in the following manner. In the early morning, prior to drawing blood from the participants:

1. Check to make sure the blood collection tray is properly equipped. Every item on the checklist must be ready before proceeding.

2. Check supply of Vacutainer tubes.

3. Check that the microvial rack is filled.

4. Perform and record QC check on refrigerator temperature (4°C $\pm$ 2°C).

5. Perform and record QC check on freezer temperature (-70°C $\pm$ 10°C).

At participant arrival:

1. Enter the participant's HEIRS ID Number on the Lab Test Request Form when the participant arrives for the visit. At the completion of specimen collection and processing, the entire original Lab Test Request Form is sent to the Central Laboratory, and a copy is kept on file at the Field Center.

2. Place a LABID label on the Lab Test Request Form. Complete the other fields on the form.

3. Place a LABID label on the Clinical Assessment form. Match it to the participant's HEIRS ID.

4. Place a LABID label on each of the six blood collection tubes.

5. Check that Quality Control tubes are prepared and labeled, if needed (see Quality Control section of this manual for details).

6. The venipuncture procedure may now proceed.

3. VENIPUNCTURE

3.1 Precautions for Handling Blood Specimens

Handle all specimens as potentially infectious for laboratory workers. OSHA rules mandate that technicians must always wear disposable protective gloves when collecting and processing specimens.

Use 0.5% sodium hypochlorite (household bleach diluted 1:10) to clean up any spills of blood, plasma, or serum.

OSHA regulations require that all needles and sharp instruments be discarded into puncture resistant containers.

Avoid formation of potentially infectious aerosols when removing the rubber stoppers from Vacutainer tubes. In addition to wearing protective gloves, hold a piece of gauze over the stopper while slowly removing it from the tube.

Place all used Vacutainer tubes and blood-contaminated products in biohazard bags for proper disposal.

3.2 Phlebotomy Room

The blood drawing takes place in an isolated room or in an area where participants are separated by room dividers.

3.3 Participant Preparation

Informed consent must be obtained from the participant before drawing blood. This procedure is followed to ensure that the participants understand the purpose of blood drawing and the possible complications of venipuncture.

Blood drawing is standardized to the sitting position. It is difficult to standardize the length of time that a person is in the sitting position prior to venipuncture, but allow enough time for the participant to relax before the venipuncture takes place.

Give the participant enough time to feel comfortable after the blood collection, as well. In many cases the most memorable part of the experience for participants will be the contact with the technicians who draw the blood and their general attitude and competence.

HANDLING PARTICIPANTS WHO ARE EXTREMELY APPREHENSIVE ABOUT HAVING BLOOD DRAWN: Do not under any circumstances force the participant to have blood drawn. It may help to explain to the participant that the blood drawing is designed to be as nearly painless as possible. It may also be helpful to have the participant relax in the blood drawing chair just so the phlebotomist can check the veins in the participant's arms, without actually drawing blood. If the participant is very anxious, he/she may lie down during the blood collection.

3.4 Venipuncture

Before applying the tourniquet, screw the 21-gauge needle into the plastic Vacutainer tube guide.

With jacket or sweater removed, have the participant sit upright with the sleeves rolled up to expose the antecubital fossa (elbow). The preferred arm to draw from is the left arm. The right arm should be used only if blood collection is not possible from the left

arm. This does not mean you must stick the left arm. Only do so if an adequate vein is apparent.

PRECAUTIONS WHEN USING A TOURNIQUET: The tourniquet should be on the arm for the shortest time possible. Never leave the tourniquet on for longer than two minutes. To do so may result in hemoconcentration or a variation in blood test values. If a tourniquet must be applied for preliminary vein selection, and it remains on the arm for longer than two minutes, it should be released and reapplied after a wait of two minutes. Instruct the participant that he/she should not clench their fist prior to the venipuncture. Doing so could cause fluctuations in the results in several of the analytes being measured. If the participant has a skin condition, put the tourniquet over the participant's shirt or use a piece of gauze or paper tissue so as not to pinch the skin. Wrap the tourniquet around the arm 3 to 4 inches (7.5 to 10.0 cm) above the venipuncture site.

Identify the vein, then cleanse the venipuncture site.

1. Remove alcohol prep from its sterile package.

2. Cleanse the vein site with the alcohol prep using a circular motion from the center to the periphery.

3. Allow the area to dry to prevent possible hemolysis of the specimen and a burning sensation to the patient when the venipuncture is performed.

4. If venipuncture becomes difficult, the vein may need to be touched again with your hand. If this happens, cleanse the site again with alcohol.

Perform venipuncture.

1. Grasp the participant's arm firmly, using your thumb to draw the skin taut. This anchors the vein. The thumb should be 1 or 2 inches (2.5 or 5.0 cm) below the venipuncture site.

2. With the needle bevel upward, enter the vein in a smooth continuous motion.

3. Make sure the participant's arm is in a flat or downward position while maintaining the tube below the site when the needle is in the vein. It may be helpful to have the participant make a fist with the opposite hand and place it under the elbow for support. DO NOT HAVE THE PARTICIPANT MAKE A FIST IN THE HAND OF THE ARM FROM WHICH BLOOD IS TO BE DRAWN.

4. Once the needle appears to be in the vein insert tube #1 into the plastic vacutainer tube guide. Grasp the flange of the tube guide and push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle. The tube should begin filling with blood.

5. Once the draw has started, do not change the position of a tube until it is withdrawn from the needle. Release the tourniquet as soon as the blood begins to flow into the first tube.

6. Keep a constant, slight forward pressure on the end of the tube. This prevents release of the shutoff valve and stopping of blood flow.

7. Fill each Vacutainer tube as completely as possible; i.e., until the vacuum is exhausted and blood flow ceases. If a Vacutainer tube fills only partially, remove the tube and attach another without removing needle from vein.

8. When the blood flow into the collection tube ceases, remove the tube from the holder. The shutoff valve covers the point, stopping blood flow until the next tube is inserted (if necessary). Tubes #3, #4, #5, and #6 should be gently inverted 8 - 10 times immediately following removal of the tube from the tube guide, then placed into the room temperature rack.

If a blood sample is not forthcoming, the following manipulations may be helpful.

1. Turn needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein.

2. Move needle slightly in hope of entering vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on either arm. The same technician should not attempt a venipuncture more than twice. If a third attempt is necessary, a different phlebotomist should attempt the venipuncture.

3. Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. If the tourniquet is a Velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than two minutes at a time.

At the conclusion of the blood draw:

1. Remove the last collection tube from the Vacutainer tube holder.

2. If not already done, release the tourniquet.

3. <u>Lightly</u> place clean gauze over the venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard the butterfly needle, adapter and Vacutainer tube holder into a needle box. DO NOT ATTEMPT TO RECAP NEEDLES! Have the participant hold the gauze pad firmly for one to two minutes to prevent a hematoma.

4. If blood flow stops before collecting all tubes, repeat the venipuncture, collecting only the unfilled tubes from the previous attempt.

Bandaging the arm.

1. Under normal conditions:

    a. Slip the gauze pad down over the site, continuing mild pressure.

    b. Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.

2. If the participant continues to bleed:

    a. Apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops.

    b. Wrap a gauze bandage tightly around the arm over the pad.

    c. Tell the participant to leave the bandage on for at least 15 minutes.

PRECAUTIONS - WHEN A PARTICIPANT FEELS FAINT OR LOOKS FAINT FOLLOWING THE BLOOD DRAWING:

1. Have the person remain in the chair. If necessary, have him/her lie on the floor with their legs elevated. Use of a transfer belt may be indicated in this situation.

2. Take an ampule of smelling salts, crush it, and wave it under the person's nose for a few seconds.

3. Provide the person with a basin if he/she feels nauseous.

4. Have the person stay seated or lying down until he/she feels better.

5. Have someone stay with the person to prevent them from falling and injuring themselves if they should faint.

6. Place a cold wet cloth on the back of the person's neck or on their forehead.

7. Once the episode has passed, some fruit juice may be given to the participant in order to counteract any possible hypoglycemia due to their pre-clinic visit fast.

8. If the person continues to feel sick, take a blood pressure and pulse reading. Contact a medical staff member, who will advise you on further action.

3.5 Specimen Delivery to Processing Area

Send the six labeled collection tubes, along with all extra LABID labels, to the processing area. The labels will be used on the storage microvials and shipping forms.

4. BLOOD PROCESSING

Processing of the various blood samples is divided into 3 stages.

4.1 Stage One: Immediate Processing

At the conclusion of venipuncture, refrigerate tube #4 until packing for shipment and keep the other tubes at room temperature.

Tubes #1 and 2 remain at room temperature for thirty minutes to allow the blood to clot (blood at 4°C clots extremely slowly). Set a timer for 30 minutes as a reminder to centrifuge this tube.

4.2 Operating the Centrifuge

Refer to Centrifuge Operating Manual for specific operating and balancing instructions. In order to achieve a 1100 - 1300 x *g* centrifugal force within the centrifuge, the corresponding revolutions per minute (RPM) will vary from centrifuge to centrifuge depending on radius of the centrifuge's rotor. Consult the centrifuge's operating manual for the appropriate RPM for each centrifuge.

4.2.1 Centrifugation

1. As soon as possible after the 30 minutes timer goes off, and not longer than 45 minutes after blood collection, place tubes#1, #2, and #3 in a centrifuge trunion. Balance the centrifuge, then spin at 1100 - 1300 x *g* for 15 minutes at room temperature.

2. Wait for centrifuge to come to a complete stop. Remove the tube from the centrifuge as soon as possible. Proceed to stage two processing.

4.3 Stage Two: Blood Processing

1. Remove tubes #1, #2, and #3 from the centrifuge and place them near the rack holding microvials.

2. Label eight 2.0-mL microvials and one 8-mL vial with LABID labels matching the LABID on the collection tubes.

3. Remove the stoppers from the collection tubes # 1 and #2. Using a plastic transfer pipette, aliquot all of the serum equally into the six microvials. Fasten red screw

caps on each of the microvials and leave them in the rack at room temperature until processing of tube #3 is complete.

4. Remove the stopper from the collection tubes # 3. Using a plastic transfer pipette, and being careful not to disturb the cell layer, remove the clear plasma supernatant. The pipette tip should not get any closer than one-half inch from the cells. Place the plasma in two 2.0-mL microvials. Discard any remaining plasma that does not fit into two microvials into a biohazard waste container. Screw yellow caps onto these microvials and leave them in the rack at room temperature.

5. Using a plastic transfer pipette, remove the packed cells and one-half inch of plasma from the lavender-topped blood collection tubes #3 and transfer it into the empty 8-mL screw top vial. Fasten a large purple screw cap onto the 8-mL vial and leave it in the rack.

6. Re-stopper sample collection tubes #1, #2, and #3 and discard them in a biohazard waste bag.

7. Place all of the completed microvials in -70ºC freezer (see 4.4 Freezing).

8. When a specimen set is placed into the freezer, affix the corresponding LABID label onto the Shipping Contents Sheet (Appendix IV). Please return any remaining labels to the Central Laboratory with tubes #5 and #6..

4.4 Freezing

When tube #1, #2, and #3 have been aliquotted into their respective microvials and replaced in the rack, the entire rack is placed upright in the -70°C freezer for a minimum of 30 minutes. Samples should be placed into the freezer within 2-3 hours after venipuncture time. Samples must be thoroughly frozen before packaging them for storage and shipping.

5. STORAGE AND SHIPPING

5.1. Storage

1. Tube #4 is stored at refrigerated temperature until it is packaged for same day shipment.

2. Tubes #5 and #6 are stored at room temperature until they are packaged for same day shipment.

3. Place all of the frozen specimen vials from a single participant into a 4” x 6” zip-seal storage bag. Check again to make sure all tubes are numbered. Press the air out of the bag and seal. Place this bag in the Central Laboratory box in the -70°C freezer

and do not remove it until the time of shipment. This shipment is prepared weekly or biweekly depending on number of participants.

5.2 Shipping

Tubes #4, #5, and #6 are shipped to the Central Laboratory on the same day of collection via overnight courier. Specimens should be shipped Monday through Saturday only unless prior arrangement has been made with the Central Laboratory. (The Hawaii Field Center should ship on Monday through Thursday only.) **Do not ship on Saturdays or the day before a holiday.**

All frozen specimens collected and stored are shipped to the Central Laboratory weekly or bi-monthly depending on the number of samples. These shipments must be made via overnight courier on Monday through Thursday. (The Hawaii Field Center should ship on Monday through Wednesday only.) **Do not ship on Saturdays or the day before a holiday.**

5.2.1 Packaging Instructions for Tube #4

SHIPPING MUST OCCUR ON DAY OF SPECIMEN COLLECTION.

1. Place tubes #4 into a foam tube holder. Assemble the foam tube holder and slide it into its cardboard sleeve. Close the sleeve containing the holder, and place it into a ziplock bag. Seal the bag. If specimens are collected from more than one participant, additional tube holders can be used. Two participant samples can be placed in one 5-or 3-tube holder. Multiple tube holders can be included in the same shipping box as described below.

2. To be included in specimen shipment box to HEIRS Central Laboratory:
   a. Blood specimens in bagged foam tube holders
   b. Frozen gel packs
   c. Packing material (crumpled paper, etc.) to occupy space in box
   d. Shipping inventory list (Appendix III)
   e. Test Request Form

3. Place the paper Test Request Forms and shipping forms (Appendix III) on top of the packing material.

4. Seal outer box tightly with packing tape. Affix a label stating “Human non-infectious diagnostic specimens, not restricted. Packed in compliance with IATA packaging instruction 650” or a biohazard label to outside of box.

5. Address the box and contact Federal Express for pickup.

5.2.2 Packaging Instructions for Tubes #5 and 6

SHIPPING MUST OCCUR ON DAY OF SPECIMEN COLLECTION.

1. Keep tubes sealed to preserve sterility.

2. Place tubes #5 and #6 into a foam tube holder. Assemble the foam tube holder and slide it into its cardboard sleeve. Close the sleeve containing the holder, and place it into a ziplock bag. Seal the bag. If specimens are collected from more than one participant, additional tube holders can be used. Two participant samples can be placed in one 5-tube holder. Multiple tube holders can be included in the same shipping box as described below.

3. To be included in specimen shipment box to HEIRS Central Laboratory:
   a. Blood specimens in bagged foam tube holders
   b. Room temperature refrigerant pack
   c. Packing material (crumpled paper, etc.) to occupy space in box
   d. Shipping inventory list (Appendix III)
   e. Remaining Lab ID labels

4. Seal shipping box with packing tape. Affix a label stating “Human non-infectious diagnostic specimens, not restricted. Packed in compliance with IATA packaging instruction 650” or a biohazard label to outside of box.

5. Address the box and contact Federal Express for pickup.

5.2.3 Packaging Instructions for Frozen Microvials

The bags of frozen specimens are packed and shipped in styrofoam boxes. Packaging instructions are as follows:

1. Place a layer of dry ice on the bottom of the styrofoam box.

2. Put half of the bags of specimens into the styrofoam box on top of the dry ice.

3. Layer more dry ice on top of and around the sample bags.

4. Put the remaining specimen bags into the styrofoam box on top of the dry ice.

5. Layer more dry ice on top of and around the sample bags. The amount of dry ice in the shipping box should total at least five pounds.

6. Place packing material (e.g. bubble wrap) on top of the dry ice to fill the box.

7. Place the paper shipping forms on top of the packing material. The shipping forms and instructions are shown in Appendix III.

8. Seal the outer box tightly with strapping tape. Affix a label stating “Human non-infectious diagnostic specimens, not restricted. Packed in compliance with IATA packaging instruction 650” or a biohazard label to outside of box.

9. Address the box and contact Federal Express for pickup.

10. If necessary, more than one box may have to be shipped per week.

5.2.2 Mailing Instructions

All shipping containers are sent to the Central Laboratory by overnight courier (e.g. Federal Express) to ensure receipt within 24 hours (48 hours from Hawaii). The empty styrofoam containers are returned to the Field Centers via UPS or US mail.

Containers shipped to the Central Laboratory are addressed as follows:

HEIRS Central Laboratory
Fairview-University Medical Center
Room L275 Mayo
420 Delaware Street S.E.
Minneapolis, MN 55455
Telephone: (612) 273-3318 (office)
Telephone: (612) 273-3645 (lab)
FAX: (612) 273-3489
Email: grynder1@fairview.org

6. QUALITY CONTROL

6.1 Venipuncture and Equipment Records

It is very important that the quality control records of the procedures and the equipment be properly maintained.

The Test Request Form serves as a record of the blood collection procedure.

For the equipment, daily records should be kept on all refrigerators and freezers. See Appendix V for a sample form. In addition, the actual speed of the centrifuge needs to be checked and recorded annually with a tachometer. A sample Quality Control Checklist is enclosed in this manual (see Appendix V). The local blood processing certifier will fill out this sheet monthly, certifying that daily checks have been performed properly and describing any problems in this area. The Monthly Quality Control Checklists should be kept in a permanent file in the Field Center.

6.2 Quality Control Duplicate Blood Samples

As part of the overall quality control program for laboratory analyses, duplicate specimens are sent to the laboratory, with one half of each specimen pair sent under

the participant's regular HEIRS LAB ID number, and the other half under a Quality Control Phantom Participant (QC) HEIRS ID number and LAB ID number. The QC LAB ID numbers are not distinguishable from other LAB ID numbers so that this forms a blinded external quality control program monitoring measurement variability.

To reduce the burden upon HEIRS participants, no one person is asked to contribute sufficient extra blood to make a complete set of duplicates for all tests. Instead, extra blood is drawn from two participants and sent to the Central Laboratory under 2 different "phantom" HEIRS ID numbers and QC LAB ID numbers. For data analysis, results on each laboratory measurement are matched to the appropriate participant results.

Every 20th HEIRS Laboratory ID label is highlighted with a colored marker to remind the technician that QC samples need to be collected. (The participant who is assigned the highlighted labels does not necessarily have to be one of the volunteer for the QC samples.) Only blood collection tubes #1, #2, #3 and #4 will be collected for QC samples. The first volunteer participant will donate additional red/gray stoppered tubes #1 and #2. The second volunteer participant will donate additional lavender stoppered tubes #3 and #4. When a participant is selected for the QC sample collection, select a new Lab ID number and a phantom HEIRS ID# (from the screening ID# list as is done for the screening QC samples) for these replicate samples. For the first participant who volunteers to donate a QC sample (tubes #1 and #2), place a Lab ID# label on the Clinical Assessment form 6, page 2 in the box next to "Fairview replicate" and a second label with the same Lab ID# on the Lab Test Request Form used for the first QC donor. Also, place a phantom HEIRS ID# on this Lab Test Request Form and enter "HEIRSX" for the acrostic. For the second participant who volunteers to donate a QC sample (tubes #3 and #4), place a different Lab ID# label on their Clinical Assessment form 6, page2 in the box next to "Fairview replicate" and a second label with the same Lab ID # on a Lab Test Request Form used for the second QC donor. Also, place a different phantom HEIRS ID# on this Lab Test Request Form and enter "HEIRSX" for the acrostic. Example forms for the QC process are shown in appendix IX.

6.2.2 Preparation for Drawing and Processing QC Samples

Blood Drawing Tubes: Each morning of the day(s) designated for QC collection, the blood drawing technicians prepare extra blood collection tubes for the QC samples to be drawn. Each tube is labeled with the QC ID number to be used. Remember that QC tubes #1 and #2 will get one Lab ID number and tubes #3 and #4 will get a different Lab ID number.) In addition, the technicians may wish to mark QC tubes #1, #2, #3,and #4 "QC" in a clearly visible fashion, to reduce the chance that these tubes might be mixed up with the regular blood collection tubes during processing.

Sample Aliquot Tubes: Each morning of the day(s) designated for QC collection, a separate rack is prepared for the set of QC blood tubes that the technician plans to draw that day. The rack contains all the aliquot tubes needed to process the day's quality control samples. The tubes in each rack are labeled in advance with the QC ID

numbers being used for the QC set. Care must be taken during processing that the labels on the sample aliquot tubes match the label on the QC blood collection tubes.

6.2.3 Collecting and Processing QC Blood

Selecting Participants for QC Blood Draw: Collect QC specimens from participants who have quality veins, and from whom blood is easily collected. It is conceivable that a set of QC samples will be collected from two participants on different days.

Order of QC Tubes in Relation to Regular Blood Collection: Collect the QC tube after the other tubes have been filled. A NEW NEEDLE STICK SHOULD NOT BE DONE JUST TO GET MORE BLOOD FOR A QC SPECIMEN.

Processing and Freezing QC Blood: QC blood samples are processed, stored, and shipped along with the regular blood samples. The QC sample collection tube #4 is shipped refrigerated the same day as collection along with the Test Request Form with the matching Lab ID#. Place the Test Request Form with the Lab ID# matching the serum aliquots from tubes #1 and #2 with the shipping logs for the frozen shipment.

6.3. Reporting of Results

The Central Laboratory has the responsibility for reporting results to the Coordinating Center and to fax the results to the appropriate Field Center. When a participant has volunteered to submit and extra sample for quality control, both sets of results will be reported to the Field Center and the Coordinating Center. Only the results from the sample collected using the participants correct HEIRS ID and associated Lab ID# should be reported to the participant. All test results are transmitted to the Coordinating Center via FTP. This transmission occurs twice per week.

7. TRAINING PROCEDURES

7.1 Technician Training and Evaluation

The technician must study the HEIRS Specimen Collection and Processing Manual and watch several participant samples being processed. Then the technician may proceed to a mock drawing and mock processing of samples, without performing any actual venipuncture. Mock venipuncture is performed with the Vacutainer system. A piece of latex tubing with a knot in one end leading to a glass of water is used as a target vein. Practice tubes are collected in the correct order, then placed at their proper positions. The sample is processed from start to finish exactly as if real blood were being used. Each technician performs a minimum of two mock draws from beginning to end. Although the mock draws take time, they provide hands-on experience and allow the technician to become comfortable with the procedures before proceeding to live participants.

At this point the technicians are ready to practice on live volunteers. The technicians practice at least once with just one volunteer at a time and again process the blood entirely by themselves from start to finish. If the technicians do not feel comfortable, they can repeat the process with dummy tubes. If enough volunteers are available, it may be beneficial to repeat this several times. Any questions or problems that the technicians have must be solved before the technicians proceed to drawing the HEIRS participants. Before the technicians draw blood from any HEIRS participant, they must take and pass the practical and written tests included at the end of this manual. After passing the tests and evaluation of their instructor, they may proceed to drawing blood from HEIRS participants.

Appendix I List of Tests

Comprehensive Clinical Exam Visit:

Analyte	HEIRS Reference Range		Units
ALT	0-40 male	0-31 female	U/L
AST	0-37 male	0-31 female	U/L
Bilirubin, Total*	0.0-1.0		mg/dL
Bilirubin, Direct*	0.0-0.3		mg/dL
Bilirubin, Indirect*	0.0-0.7		mg/dL
CRP	0-0.5		mg/dL
GGT	11-51 male	7-33 female	U/L
Glucose	60-115		mg/dL
Haptoglobin*	300-200		mg/dL
HEIRS Study Iron Panel			
Ferritin	See attached letter		
Iron	45-160 male	30-160 female	ug/dL
Iron Binding Capacity	228-428		ug/dL
Iron Saturation Index	15-50		Percent
Insulin (not reported to Field Center)	0-20		mIU/L
LD*	94-250		U/L
Hemogram			
WBC Count	4.0-11.0		10e9/L
RBC Count	4.4-5.9 male	3.8-5.2 female	10e12/L
Hemoglobin	13.3-17.7 male	11.7-15.7 female	g/dL
Hematocrit	40.0-53.0 male	35.0-47.0 female	Percent
MCV	78-100		fl
MCH	26.5-33		pg
MCHC	32.0-36.0		g/dL
RDW	11.0-15.0		Percent
Platelet Count	150-450		10e9/L
Differential			
% Neutrophils	40-75		Percent
% Lymphocytes	20-48		Percent
% Monocytes	0-12		Percent
%Eosiniphils	0-6		Percent
%Basophils	0-2		Percent
Abs Neutrophils	1.6-8.3		10e9/L
Abs Lymphocytes	0.8-5.3		10e9/L
Abs Monocytes	0.0-1.3		10e9/L
Abs Eosiniphils	0.0-0.7		10e9/L
Abs Basophils	0.0-0.2		10e9/L

Reticulocyte count*	0.4-2.5	Percent
Hemoglobin Evaluation*		
Hemoglobin A1	94.3-98.5	Percent
Hemoglobin A2	1.5-3.7	Percent
Hemoglobin F	0.0-2.0	Percent
Hemoglobin S	0.0-0.0	Percent
Hemoglobin C	0.0-0.0	Percent
Hemoglobin E	0.0-0.0	Percent
Hemoglobin Other	0.0-0.0	Percent
HbsAg*	Negative	
HCV antibody*	Negative	
Other polymorphism detections to be determined	NA	
Lymphocyte cryopreservation.	NA	

*Hepatitis B surface antigen and hepatitis C antibody will be performed only on those participants with ALT results above the gender-specific reference range. Reticulocyte count, hemogobin evaluation (identification and quantitation), haptoglobin, LD, bilirubin (total, direct and indirect) will be done if hemoglobin or MCV is below the reference range.

Family Study Visit:

Testing is the same list as Comprehensive Clinical Exam. In addition, C282Y and H63D genotyping will be performed if not already analyzed and a genome screen will be performed.

Appendix II. Equipment and Supplies

Supplies to be obtained by Field Center

Supplier	Catalog no. Usage/week	Description	
Sarstedt	72.609	Microvials 500/pk	
"	65.716.003	Red Screw Caps 1000/pk	
"	65.716.002	Yellow Screw Caps 1000/pk	
"	60.542.530	8-mL storage vial	
"	65.176.007	Purple screw cap (lrg)	
Fisher	02-665-21	Needles, 21G x 1 ½ in. 100/pk	
"	06-669-62	Alcohol Swabs 4000/cs	
"	17-986-78F	Gauze Sponges 200/pk	
"	17-442-3B	Band Aids 100/pk	
"	22-035365	Tourniquets 250/pk	n/a
"	02-658-210	Vacutainer Tube Holders 1000/cs	
"	13-711-9A	Transfer Pipettes 500/pk	
"	01-816A	Freezer Bags 4" x 6"	
		Dry Ice (approximately 9 lbs./shipping box)	
"	06-669-69	PDI Ammonia Inhalant 100/pk	n/a
		Vacutainer Tubes 100/pk	
"	02-657-11	Serum Separator red/gray top, BD#366510	
"	02-683-84	10 mL, EDTA, lavender top, BD#366457	
"	05-669-47	Microvial Rack	n/a
"	15-252	Blood Collection Trays	n/a
"	15-105-5	Refrigerator/Freezer Thermometers	n/a
"	02-037SD	Harvard Trip Balance (Ohaus 1550SD)	n/a
"	06-662-3	Timer - 3 channel digital	n/a
Officemax.com	07018194	Bubble wrap	n/a
Polyfoam Packers		*	
	Styrofoam shipping box		
"	409	Five tube mailer	
"	410	Five tube mailer sleeve	
"	364	Three tube mailer	
"	365	Three tube mailer sleeve	
UAL Medical Prod	BH302CL	Biohazard labels 320/roll	

*Recommendations for shipping boxes.
All boxes are from Polyfoam Packers, (800) 323-7442, www.polyfoam.com.

Box #324. I.D. 8 x 6 x 11.5 (L x W x H), 1.5 inch wall. This box would be used for frozen CCE specimens from sites other than Hawaii.

Box #453, I.D. 12 x 12 x 11.5 (L x W x H), 2 inch wall. This box would be used for frozen specimen shipments from Hawaii. The 2 inch wall provides additional insulation for insurance against shipping delays.

Box#324 above will hold up to 4 five-tube mailers. Smaller boxes may also work (#321 or #413). These boxes have the same length and width, but their heights are 8 7/8 and 7 inches, respectively.

Equipment purchased and maintained by Field Centers:

Table-top centrifuge capable of producing 1,500 x *g*
Freezer (-70°C)
Refrigerator (4°C)

Appendix III. HEIRS Shipping Forms –Frozen Aliquots

HEIRS Central Laboratory
Fairview-University Medical Center
Specimens Room L275 Mayo
420 Delaware Street S.E.
Minneapolis, MN 55455

Contents Sheet
Frozen
Page ____ **of** ____

Ship date _____

Complete Sample:
6-red top 2.0 mL microvials
2-yellow top 2.0 mL microvials
1-purple top 8.0 mL vial

LABID	SET COMPLETE? YES	NO	MISSING VIALS #	COLOR	COMMENTS

Additional Contents

Sheet

Frozen Specimens
Page ____ of ____
Ship Date

_____

LABID	SET COMPLETE? YES	NO	MISSING VIALS #	COLOR	COMMENTS

HEIRS Shipping Forms –Refrigerated 10-mL EDTA Tube

HEIRS Central Laboratory
Fairview-University Medical Center
Specimens Room L275 Mayo
420 Delaware Street S.E.
Minneapolis, MN 55455

Contents Sheet
Refrigerated
Page ____ **of** ____
Ship date _____

Complete Sample:
1-10-mL EDTA tube (#4)

LABID COMMENTS

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Additional Contents

Sheet

Refrigerated

Specimens

Page ____ of ____
Ship Date

_____

LABID COMMENTS

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

HEIRS Shipping Forms –Ambient Temperature Specimens

HEIRS Central Laboratory
Fairview-University Medical Center
Specimens Room L275 Mayo
of ____
420 Delaware Street S.E.
Minneapolis, MN 55455

Contents Sheet
Ambient Temp.
Page ____

Ship date _____

Complete Sample:
2-CPT Tubes (#5 and #6)

LABID	SET COMPLETE? YES	NO	COMMENTS

Additional Contents

Sheet

Ambient Temp.

Specimens

Page ____ of ____
Ship Date

_____

LABID	SET COMPLETE? YES	NO	MISSING VIALS #	COLOR	COMMENTS

CONTENTS SHEET INSTRUCTIONS

The contents sheets list the complete inventory of specimens in a shipment. The original form is sent to the Central Laboratory with the specimen shipment, and a copy is filed at the Field Center. More than one contents sheet may be used in each shipment, depending on the number of specimens enclosed. The number of pages attached and each page number are filled in at the top of the contents page (e.g. "page 1 of 5"). This form is filled out at the Field Center as the specimens are collected and stored. This form must be checked against the specimens when packed for shipment. Record the date of shipment.

The LABID number is entered in the left hand column of the contents sheet. This is most easily done by attaching one of the adhesive LABID labels in the space provided. This should be done when the processed specimens are placed into the freezer. It is suggested that a second person check the Contents Sheet LABIDs against the LABIDs on the vials to discover any errors.

The tubes comprising a complete set are listed in the upper left hand corner of the sheet. Under the category SET COMPLETE?, YES or NO should be marked for each participant to indicate whether the correct number of tubes has been shipped. If there is some deviation from the correct count, "NO" should be marked, and a description of the problem should follow in the column headed MISSING VIALS. The number of missing tubes and the color of their caps should be recorded here.

COMMENTS on the quality of the specimens upon receipt are recorded at the Central Laboratory.

Appendix IV. HEIRS Daily Temperature Record

HEIRS
DAILY TEMPERATURE RECORD

DATE Mo/Da/Yr	Freezer	Refrig	Room	Initials	DATE Mo/Da/Yr	Freezer	Refrig	Room	Initials

Appendix V. HEIRS Monthly Equipment Quality Control Checklist

CENTER _____
DATE _____
TECHNICIAN _____
ID NUMBER _____

	(S)atisfactory/(U)nsatisfactory	Comments
1. Daily QC records		
Refrigerator temperature	___	
Freezer temperature	___	
Room temperature	___	_____
2. Annual QC records		
Centrifuge tachometer check	___	
3. Equipment and Supplies		
Timer	___	
Vacutainer tubes	___	_____
Venipuncture needles	___	_____
Vacutainer tube guides	___	_____
Alcohol swabs	___	
Gauze sponges	___	_____
Tourniquets	___	_____
Bandages	___	_____
Smelling salts	___	
Microvials	___	_____
Red/Yellow/Purple screw caps	___	
Transfer pipets	___	_____
Freezer bags	___	_____
Shipping boxes	___	_____
Biohazard labels	___	_____

Appendix VI. HEIRS Venipuncture and Processing Procedures Certification Checklist

VENIPUNCTURE	Satisfactory/ Unsatisfactory	Comments
1. Labels checked	___	_____
2. Participant prepared and procedure explained.	___	_____
3. Lab Test Request Form filled.	___	_____
4. Tourniquet application and release	___	_____
5. Venipuncture technique	___	_____
6. Tube collection sequence	___	_____
7. Inversion technique	___	_____
8. Tube incubation location	___	_____
9. Stasis obtained	___	_____
10. Needle disposal	___	_____

PROCESSING		
1. Knowledge of centrifuge operation	___	_____
2. Aliquotting supply set-up	___	_____
3. Stage I tube spin	___	_____
4. Stage II aliquotting	___	_____
5. Stage III tube spin	___	_____
6. Vials sealed	___	_____
7. Final processing stage	___	_____
8. Freezer organization	___	_____

9. Time constraints ___ _____

10. Disposal of contaminated supplies ___ _____

PACKAGING AND SHIPPING

1. Specimens bagged ___ _____
2. Adequate dry ice used in shipping ___ _____
3. Shipping paperwork ___ _____
4. Containers correctly labeled for shipping ___ _____

MISCELLANEOUS

1. QC Procedure ___ _____

PRACTICAL EXAM FOR HEIRS BLOOD DRAWING TECHNICIAN

1. Organize the blood collection tray for collection of the two blood collection tubes. Correctly label all forms and collection tubes.

2. Specify which tube(s) remain at room temperature after collection.

3. Remove the appropriate tubes from the tray, balance them and place them in the centrifuge. How long should they spin? At what speed?

4. Set up a rack with the appropriate number and order of microvials. Indicate the colors of screw caps and the types of specimen put into these tubes. Correctly label all microvials.

5. Correctly dispense the different specimen types to their storage microvials.

6. Organize the color-capped sample tubes and prepare them for shipment.

7. Execute the quality control procedure for collection and processing of blind duplicates.

WRITTEN EXAM FOR HEIRS BLOOD DRAWING TECHNICIAN

1. When handling biological specimens, what type of protective apparel must always be worn? _____

2. What is the recommended solution for use in cleaning an area where blood has spilled? _____

1. List three steps to take if a participant becomes faint during the blood collection procedure:

   1. _____

   2. _____

   3. _____

4. Is it acceptable for the participant to make a fist in the hand of the arm from which the blood specimen is being collected? If so, when?
   _____

   _____
   _____

5. During a typical week, how many **HEIRS** participants will have additional blood specimens collected to be used as part of the phantom duplicate?

   a) 5
   b) 4
   c) 2
   d) 0

6. From which tube is the buffy coat used?

   a) None
   b) #1
   c) #2
   d) #3

7. How long should tube #1 sit at room temperature before centrifugation?

   a) 5 minutes
   b) 30 minutes
   c) 2 hours
   d) No waiting time required

8. Why is this step (un)necessary?_____

9. For what type of tests will the buffy coat be used?

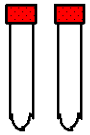
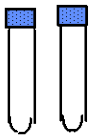
   a) Chemistry
   b) Lipid
   c) Genetic
   d) Coagulation

10. When is the tourniquet removed?

   a) after tube #1 fills
   b) after tube #2 fills
   c) prior to beginning the venipuncture

Appendix VIII. Blood Processing Flow Chart

HEIRS Central Laboratory

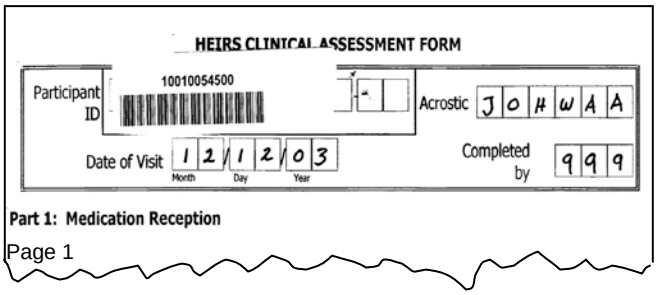
BLOOD COLLECTION	STAGE 1 immediate processing & centrifugation	STAGE 2 Blood Processing	STORAGE
Tubes **#1 & 2**  10-mL red/gray top tube	Room Temperature rack for 30 - 45 min; then centrifuge for 15 min at 1100-1300 *x g*	Transfer all serum into six 2-mL microvials. Store at Room Temperature until frozen 	Store vials at -70°C
Tube **#3**  10-mL lavender top tube invert 8-10 times	Room Temperature rack then centrifuge for 15 min at 1100-1300 *x g*	Transfer supernatant equally into two 2-mL microvials  Transfer packed cells from tube #3 into one 8 mL vial 	Store vials at -70°C
Tube **#4**  10-mL lavender top tube invert 8-10 times	Store refrigerated until shipment		Store tube refrigerated
Tubes **#5 & 6**  10-mL blue/black CPT tubes invert 8-10 times	Store at room temperature until shipment		Store tubes at room temperature

Shipping to the Central Laboratory: vials stored at -70°C -->ship on dry ice weekly or bimonthly
Tube #4 at 4°C --> ship same day as collection, include Lab Request Form
Tubes #5 & 6 at ambient temperature --> ship same day as collection, include extra Lab ID labels
(Complete HEIRS Shipping Forms and include in each shipment.)

Questions regarding this protocol can be answered by contacting the Central Laboratory at 612-273-3645

Appendix IX. Quality Control Example Forms
HEIRS Participant QC Volunteer 1

1. Complete Clinical Assessment Forms Form 1



HEIRS CLINICAL ASSESSMENT FORM

Participant ID: 10010054500

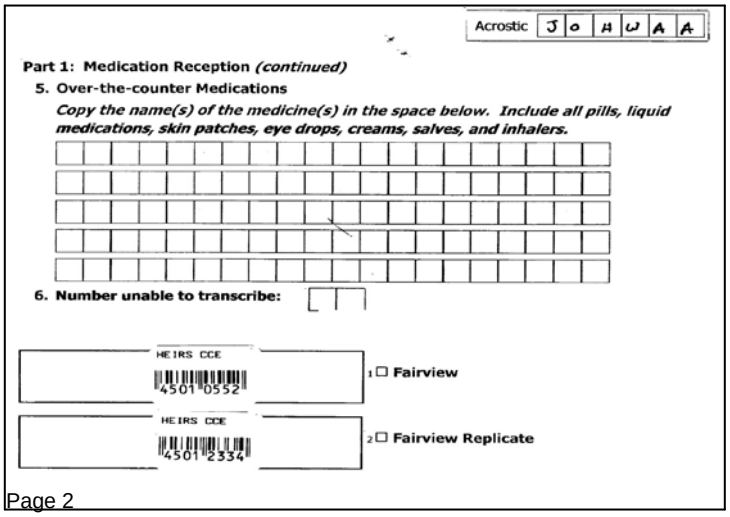
Acrostic: J O H W A A

Date of Visit: 1 2 / 1 2 / 0 3 (Month / Day / Year)

Completed by: 9 9 9

Part 1: Medication Reception

Page 1



Acrostic: J O H W A A

Part 1: Medication Reception *(continued)*

5. Over-the-counter Medications

Copy the name(s) of the medicine(s) in the space below. Include all pills, liquid medications, skin patches, eye drops, creams, salves, and inhalers.

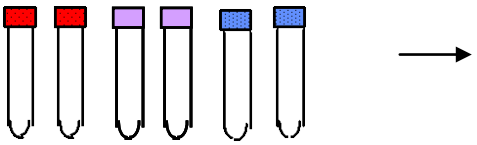
6. Number unable to transcribe:

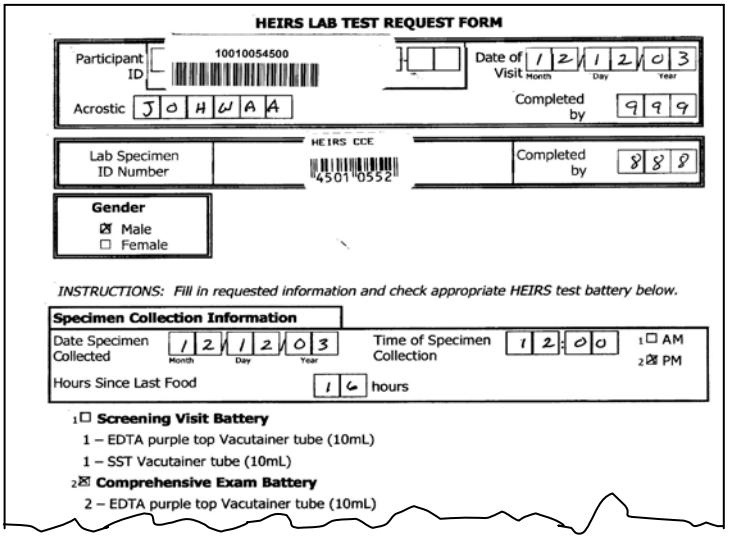
HEIRS CCE 4501 0552 — 1 ☐ Fairview

HEIRS CCE 4501 2334 — 2 ☐ Fairview Replicate

Page 2

2. Collect Blood and Complete Lab Request Form





HEIRS LAB TEST REQUEST FORM

Participant ID: 10010054500

Acrostic: J O H W A A

Date of Visit: 1 2 / 1 2 / 0 3 (Month / Day / Year)

Completed by: 9 9 9

Lab Specimen ID Number: HEIRS CCE 4501 0552

Completed by: 8 8 8

Gender: ☒ Male ☐ Female

INSTRUCTIONS: Fill in requested information and check appropriate HEIRS test battery below.

Specimen Collection Information

Date Specimen Collected: 1 2 / 1 2 / 0 3 (Month / Day / Year)

Time of Specimen Collection: 1 2 : 0 0 — 1 ☐ AM 2 ☒ PM

Hours Since Last Food: 1 6 hours

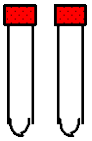
1 ☐ **Screening Visit Battery**

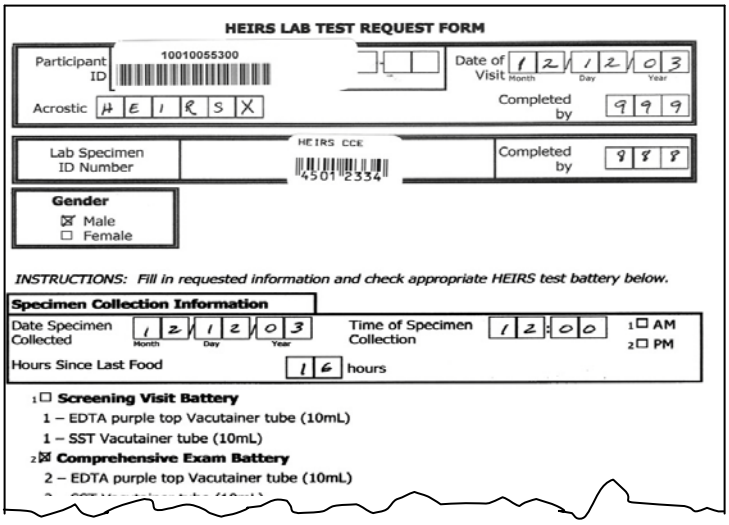
1 – EDTA purple top Vacutainer tube (10mL)

1 – SST Vacutainer tube (10mL)

2 ☒ **Comprehensive Exam Battery**

2 – EDTA purple top Vacutainer tube (10mL)





HEIRS LAB TEST REQUEST FORM

Participant ID: 10010055300

Acrostic: H E I R S X

Date of Visit: 1 2 / 1 2 / 0 3 (Month / Day / Year)

Completed by: 9 9 9

Lab Specimen ID Number: HEIRS CCE 4501 2334

Completed by: 8 8 8

Gender: ☒ Male ☐ Female

INSTRUCTIONS: Fill in requested information and check appropriate HEIRS test battery below.

Specimen Collection Information

Date Specimen Collected: 1 2 / 1 2 / 0 3 (Month / Day / Year)

Time of Specimen Collection: 1 2 : 0 0 — 1 ☐ AM 2 ☐ PM

Hours Since Last Food: 1 6 hours

1 ☐ **Screening Visit Battery**

1 – EDTA purple top Vacutainer tube (10mL)

1 – SST Vacutainer tube (10mL)

2 ☒ **Comprehensive Exam Battery**

2 – EDTA purple top Vacutainer tube (10mL)

NOTE: Send this QC Lab form with the frozen serum aliquots.

HEIRS Participant QC Volunteer 2

3. Complete Clinical Assessment Forms Form 2

HEIRS CLINICAL ASSESSMENT FORM

Participant ID 10010054700 Acrostic B U R S H R

Date of Visit 12/12/03 (Month/Day/Year) Completed by 999

Part 1: Medication Reception

Page 1

Acrostic B U R S H R

Part 1: Medication Reception *(continued)*

5. Over-the-counter Medications

Copy the name(s) of the medicine(s) in the space below. Include all pills, liquid medications, skin patches, eye drops, creams, salves, and inhalers.

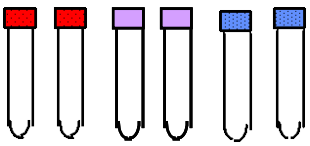
6. Number unable to transcribe:

HEIRS CCE 4500 0003 1☐ Fairview

HEIRS CCE 4501 0019 2☐ Fairview Replicate

Page 2

4. Collect Blood and Complete Lab Request Form



HEIRS LAB TEST REQUEST FORM

Participant ID 10010054700 Date of Visit 12/12/03 (Month/Day/Year)

Acrostic B U R S H R Completed by 999

Lab Specimen ID Number HEIRS CCE 4500 0003 Completed by 888

Gender ☐ Male ☒ Female

INSTRUCTIONS: Fill in requested information and check appropriate HEIRS test battery below.

Specimen Collection Information

Date Specimen Collected 12/12/03 (Month/Day/Year) Time of Specimen Collection 11:00 1☒ AM 2☐ PM

Hours Since Last Food 16 hours

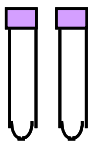
1☐ **Screening Visit Battery**

1 – EDTA purple top Vacutainer tube (10mL)

1 – SST Vacutainer tube (10mL)

2☐ **Comprehensive Exam Battery**

2 – EDTA purple top Vacutainer tube (10mL)



HEIRS LAB TEST REQUEST FORM

Participant ID 10010055200 Date of Visit 12/12/03 (Month/Day/Year)

Acrostic H E I R S X Completed by 999

Lab Specimen ID Number HEIRS CCE 4501 0019 Completed by 888

Gender ☐ Male ☒ Female

INSTRUCTIONS: Fill in requested information and check appropriate HEIRS test battery below.

Specimen Collection Information

Date Specimen Collected 12/12/03 (Month/Day/Year) Time of Specimen Collection 11:00 1☒ AM 2☐ PM

Hours Since Last Food 16 hours

1☐ **Screening Visit Battery**

1 – EDTA purple top Vacutainer tube (10mL)

1 – SST Vacutainer tube (10mL)

2☒ **Comprehensive Exam Battery**

2 – EDTA purple top Vacutainer tube (10mL)

NOTE: Please send this QC Lab slip with the CBC specimen.