HRS Documentation Report

Documentation of Biomarkers in the Health and Retirement Study

Report prepared by

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Introduction

Biomarkers refer to the general range of physiological, metabolic, biochemical, endocrine and genetic measures that can be obtained in living organisms. The term is most commonly used to refer to one-time biochemical or hematological measures made on blood or other available bodily fluids, but perhaps the term should be used for a broader range of measures.

In 2006, HRS included the following biomarkers measurements, administered in this order:

- Saliva collection for DNA extraction
- Blood spot collection for cholesterol, hemoglobin A1C, CRP and cystatin C analysis
  - (results for C-reactive protein and cystatin C are forthcoming)

This report describes the following for each of these measures:

- Rationale and key citations
- Sample description
- Measure description
- Equipment
- Protocol description
- Any special instructions

The booklet used by the interviewers to administer the 2006 physical measures and biomarkers is available on the HRS website at:
http://hrsonline.isr.umich.edu/meta/2006/core/qnaire/online/44hr06BioMarker.pdf

General Notes

Sample Selection for the Enhanced Face-to-Face Interview
A random one-half of the 2006 sample was pre-selected to complete an enhanced face-to-face interview, which included the eight measures listed above plus biomarker measurements (covered in a separate report) and the Psychosocial self-administered questionnaire. The sample was selected at the household-level to ensure that the same request was made to both members of a household. New spouses of respondents flagged to complete an enhanced face-to-face interview were also asked to do so.

The preload variable that identifies the enhanced face-to-face sample is KX090_R (located in the respondent preload file), for which a value of 3 indicates that the respondent was in the enhanced face-to-face sample. Approximately fifty percent of households with at least one living respondent were selected for the enhanced face-to-face interview across all primary sampling units (PSUs). Respondents who were selected for the enhanced face-to-face sample but were interviewed by proxy, residing in a nursing home or who declined a face-to-face interview, but agreed to be interviewed by telephone were not asked to complete the physical measures or biomarkers.

Consent Procedures
Prior to describing the individual measures, a consent form was administered by the interviewer. Respondents were asked to read and sign the form. Respondents who did not sign the consent

form were not asked to complete the measures. Separate consent forms were administered for the saliva and blood samples. Each form was introduced just prior to the measure(s) that it covered. After obtaining consent for a given component, the interviewer described the procedures to the respondent and demonstrated how the measure was conducted.

Administration Procedures
Before each measure, respondents were asked whether they understood the directions for the measurement and if they felt safe completing it. If the respondent answered no to either question, the measure was not administered. Likewise, interviewers were instructed not to administer a measure if they did not feel it was safe to complete it.

Respondents were instructed not to eat, drink, smoke, chew gum or brush their teeth during this component of the interview.

Additional eligibility criteria are listed below for each measure.

DNA Sampling and Extraction

Cells from the oral cavities of HRS participants was obtained for extraction of DNA. Over time, this will allow a wide variety of genetic studies with almost any well-defined phenotype available in the HRS data set. Past research has shown wide application in population studies.

- Garcia-Closas M, et al. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol Biomarkers Prev. 2001;10:687.
- Zheng S, et al. Whole genome amplification increases the efficiency and validity of buccal cell genotyping in pediatric populations. Cancer Epidemiol Biomarkers Prev. 2001;10:697.
- Rylander-Rudqvist T, et al. Quality and quantity of saliva DNA obtained from the self-administered Oragene method—a pilot study on the cohort of Swedish men. Cancer Epidemiol Biomarkers Prev. 2006;15:1742.

<u>Sample</u>
All respondents meeting the criteria for the enhanced face-to-face interview were eligible for this measure. Consent to complete this measure was administered prior to beginning the measure. The number of respondents for whom DNA was extracted and stored from the 2006 wave of the HRS is 6,933.

<u>Measure</u>
A saliva sample was obtained using a mouthwash technique. Saliva samples were sent to a central laboratory where they were processed, DNA was extracted and stored.

<u>Equipment</u>
A trial size bottle of original flavor Scope mouthwash
A small measuring cup (similar to those provided with liquid medications)
A collection container
Sample ID labels

Two sheets of absorbable cloth
A laboratory submission form
A small plastic bag
Latex gloves

Protocol

- The saliva barcode ID was placed on the consent form, the Saliva Authorization Form, and the collection container.
- The interviewer put on a pair of latex gloves and opened the clear plastic bag.
- One sheet of the absorbent material was placed on the respondent's table or other hard surface where the collection will be taking place (to prevent leaving any spills).
- 10 ml of mouthwash was poured into the measuring cup.
- The respondent was instructed to pour the mouthwash into his/her mouth and swish vigorously for 45 seconds without swallowing.
- The interviewer used their stopwatch and indicated to the respondent when the 45 seconds was up.
- The respondent was instructed to deposit the mouthwash into the collection container.
- A lid was placed on the collection container and it was securely closed.
- The interviewer recorded the date and time on the Saliva Authorization Form.
- The unused absorbent material was placed around the collection vial (to protect in the mailing process).
- The collection container wrapped in the absorbent material and the top copy of the Saliva Authorization Form were placed in the plastic bag. Excess air was expelled from the bag before sealing it.
- The sealed bag was placed in a padded envelope and mailed to the laboratory after the interview was completed.

Special instructions

Some respondents were unable to complete the measure for health reasons. For example, diabetics or recovering alcoholics due to the sugar alcohol in the Scope, or respondents with mouth sores or recent oral surgery. If a respondent swallowed the mouthwash, coughed or spit it out prior to finishing the measurement (and it was not deposited in the container), the measure was not repeated as the buccal cells collected would have been removed and an insufficient quantity remaining.

Blood Spots

Cholesterol

Blood cholesterol is one of the most highly studied molecules in human biology and clinical medicine. It is literally a sterol, but is often designated as a fat, or "lipid." Cholesterol does not circulate by itself in the blood, but is rather bound to proteins and real lipids, to form particles known as lipoproteins. These particles come in many shapes and sizes, but historically have been studied either as blood Total Cholesterol (TC) or three major fractions: High-Density-Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and Very-Low-Density-Lipoprotein Cholesterol (VLDL-C).

HRS has performed TC and HDL-C. TC (and LDC-C, which correlates very highly with TC) is positively associated with atherosclerotic cardiovascular disease occurrence, although the relation is not as strong among older persons. TC predicts myocardial infarction, stroke, vascular kidney disease, peripheral artery disease and many other related conditions. HDL-C, on the other hand, is the "good cholesterol," and higher blood levels generally predict lower incidence rates of vascular conditions. Some of the metabolic mechanisms for this have been worked out. For those who are not familiar with the epidemiology of TC and HDL-C, some analytical cautions are offered. TC is not sensitive to whether the participant was fasting at the time of blood extraction, but HDL-C levels are related to fasting status. Also, a given level of any blood lipid may not have the same predictive value for certain diseases in different populations for several reasons, including, lack of adjustment for other risk factors, genetic and environmental differences among populations, the long term nature history of disease development, and the impact of many lipid-lowering treatments on both blood levels and disease outcomes.

Of interest, TC is related to other behaviors and conditions in addition to cardiovascular disease, including depression and suicide, Alzheimer's disease and stress-related conditions. These relationships are very complex and difficult to unravel, but have been in the literature for a long time. Some general references for further reading:

- Evered A. Understanding cholesterol and its role in heart disease. Nurs Times. 2007;103:28-9.
- Stampfer MJ. Cardiovascular disease and Alzheimer's disease: common links. J. Intern Med. 2006;260:211-23.
- Bassand JP. Managing cardiovascular risk in patients with metabolic syndrome. Clin Cornerstone. 2006;8 Suppl 1:S7-S14.
- Coryell WH. Clinical assessment of suicide risk in depressive disorder. CNS Spectr. 2006; 11:455-61.
- Serra M, et al. Social stress and neuroactive steroids. Eur Neuropsychopharmacol 2007;17:1-11.
- Ashen, MD, et al. Clinical practice: Low HDL cholesterol levels. N Engl J Med. 2005;353:1252-60.
- Huang TL, et al. Cholesterol and lipids in depression: stress, hypothalamo-pituatary-adrenocortical axis, and inflammation/immunity. Adv Clin Chem. 2005;39:81-105.

Hemoglobin A1c

Blood glucose (sugar) levels vary in all individuals on a moment to moment basis. IN most people, among the forces that perturb blood glucose is food consumption, and these levels are almost always elevated in both Types I and II diabetes. Even in the fasting state at the same time in the morning, there will be substantial intra-individual variation. One way to summarize blood glucose over a longer period of time is to measure glycosylated hemoglobin (Hb A1c) in the blood. This refers to the fact that blood sugar binds to red blood cell hemoglobin in predictable ways; and the amount of binding is related to the “integrated” level of blood glucose over time. This summary measure covers about 120 days, which is the lifespan of the average red blood cell. HbA1c is most commonly used to monitor the level of control in diabetics, but it has used profitably in other ways: as a risk factor for diabetes-related conditions such as cardiovascular disease; as method of screening for diabetes in persons whose status is unknown; and as a general indicator of anything can potentially perturb blood glucose over time, such as altered diets, other diseases or drugs and toxins.

- Consensus Committee. Consensus statement on the worldwide standardization of the hemoglobin A1C measurement: the American Diabetes Association, European Association for the Study of Diabetes, International Federation of Clinical Chemistry and Laboratory Medicine, and the International Diabetes Federation.
  Diabetes Care. 2007 Sep;30(9):2399-400.
- Bennett CM. HbA(1c) as a screening tool for detection of Type 2 diabetes: a systematic review. Diabet Med. 2007 Apr;24(4):333-43.
- Khaw KT, et al. Glycated hemoglobin as a marker of cardiovascular risk. Curr Opin Lipidol. 2006 Dec;17(6):637-43.
- Glycated haemoglobin (HbA1c) monitoring. BMJ. 2006 Sep 16;333(7568):586-8.

Sample

The blood tests were intended for all of those who were available for the enhanced face-to-face interview. See general sampling description. Special informed consent was acquired for the blood acquisition process.

Methods of Blood Determination

The entire process of blood acquisition and determination was performed using instructions and kits from Biosafe Laboratories, Chicago, IL. Biosafe is a CLIA-certified laboratory. That is, it has been certified to provide values of sufficient accuracy for clinical use. Blood was taken by pricking the participant’s finger with a sterile lancet after cleansing the finger with an alcohol swab. Droplets of blood were expressed from the finger and directly placed on specially treated filter paper, within circles printed on the paper. There was an attempt to fill six circles, but this was not always successful. The blood spots on filter paper were then placed in special foil envelopes with a dessicant packet and then within mailing containers, and shipped to Biosafe Labs. The process is constructed so that no special temperature control is needed to preserve the values of the specimens. Repeated measures within a specific laboratory run showed a coefficient of variation of less than 4% for TC and less than 3.5% for HDL-C, and less than 7% between runs. During quality control studies, the correlation between finger prick and serum levels was 0.997 for TC and 0.940 for HDL-C.

Equipment

- Cholesterol and A1c Collection Kit
- Lab Authorization Form
- 2 Blood Collection Cards (one to be analyzed, one for storage)
- 2 Foil Blood Sample Return Bags with Desiccant;
- Lancets
- Alcohol Prep Pad
- Sterile Gauze Pad
- Adhesive Bandage
- Pre-addressed, Prepaid Mailing Envelope
- Latex Gloves

Protocol

- The interviewer placed all materials on a hard, clean, and dry surface.
- The respondent was instructed to rub their hands together or massage them to get the blood flowing to the finger tips.
- The barcode label was placed on the authorization form, the consent form, the lab authorization form and on **both** blood collection cards.
- The date and time of the blood collection was recorded on the lab authorization form, and in this booklet.
- The interviewer put on a pair of latex gloves and cleaned the respondent's finger with the alcohol prep pad. The respondent's finger was dry before proceeding.
- While holding the respondents hand firmly, the lancet was placed on the side of the pad of the respondent's middle or ring finger or the thumb.
- The lancet was pressed firmly to prick the finger. If necessary, the respondent was instructed to gently squeeze their finger from the base several times to form a large drop of blood.
- The first drop of blood was wiped away with the sterile gauze pad.
- The next large drop of blood was formed and allowed to drop onto the first circle on the blood spot card.
- Interviewers were instructed to start with the left-most circle and continue filling the spots left to right (fully filling one spot before moving on to the next). If a single drop of blood does not completely fill a circle, additional drops were added beside the first until the circle is filled. The drops should not overlap.
- If the blood "pooled" on the surface of the card, the card was gently tapped to break the surface tension of the blood and allow it to flow through to the card below.
- Interviewers were instructed to fill as many spots as possible, up to six, in the following order: the 1st and 2nd spots on the analysis card; the 1st and 2nd spots on the storage card; the 3rd spot on the analysis card, and the 3rd spot on the storage card.

- The respondent was provided with a gauze pad and a bandage once the measure was complete.
- The blood spot samples were air dried for 10 to 15 minutes and then placed in their respective foil pouches.
- Both sealed foil return bags and the top portion of the lab authorization form were placed in a prepaid mailing envelope addressed to the laboratory.
- The sample was mailed to the laboratory when the interviewer left the respondent's home.

Special instructions

Interviewers were supplied with first aid instructions to use in case the respondent's finger continued bleeding. If the respondent preferred, they could prick their own finger. Only the study materials were used to conduct this measure. A second finger prick could be carried out if the first was not adequate.