

HRS Documentation Report

Documentation of Biomarkers in the 2006 and 2008

Health and Retirement Study

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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. For many of these measures, there is a normal range, and values outside of this range can serve as an indicator of disease or as an early sign of potential disease. Clinically, these values can help evaluate response to treatment. Including evaluation of certain of these measures in social science surveys is increasingly common. As Weir (2007) notes, the addition of biomarkers to any large survey like the HRS helps to validate and add information to self-reported health, may enhance the modeling of causal pathways to health, and can reveal health characteristics which are unknown to participants or not ascertained in self-reports.

Two types of biological samples used to evaluate biomarkers have been obtained in the HRS (in 2006, 2008, 2010, and 2012) to date: blood and saliva. HRS began collecting these samples in 2006 as part of the enhanced face-to-face (EFTF) interview (see below). Saliva is used for DNA extraction, and blood is used to measure a range of other biomarkers. While there are a variety of methods for collecting blood specimens, the HRS has utilized the dried blood spot (DBS) in which participants agree to have their fingers pricked and have spots of blood dripped onto cards.

The 2006 and 2008, DBS samples have been assayed for 5 biomarkers:

- a. Total cholesterol
- b. HDL cholesterol, indicators of lipid levels
- c. Glycosylated hemoglobin (HbA1c) – an indicator of glycemic control over the past 2-3 months
- d. C-reactive protein (CRP), a general marker of systemic inflammation
- e. Cystatin C, an indicator of kidney functioning.

This report provides a rationale and key citations as well as description of the data collection including sample sizes and laboratory protocol for DNA sampling and extraction and for the blood-based biomarkers noted above. More detail on data collection methods can be found in the booklets used by the interviewers to administer the 2006, 2008, and 2010 physical measures and biomarkers portion of the EFTF interview. They are available on the HRS website at:

<http://hrsonline.isr.umich.edu/modules/meta/2006/core/qnaire/online/44hr06BioMarker.pdf>
<http://hrsonline.isr.umich.edu/modules/meta/2008/core/qnaire/online/2008PhysicalMeasuresBioMarkers.pdf>
<http://hrsonline.isr.umich.edu/modules/meta/2010/core/qnaire/online/2010PhysicalMeasuresBioMarkers.pdf>

This report also provides detailed information about the laboratories that have been used to process the 2006 and 2008 DBS samples (Appendix A) along with information on weighting,

descriptive data, and comparisons with results from the National Health and Nutrition Examination Survey (NHANES). It begins with some general notes about the EFTF interview.

Weir, David R. (2007). "Elastic Powers: The Integration of Biomarkers into the Health and Retirement Study" in Weinstein, M.; Vaupel, J.W.; Wachter, K., *Bio-Social Surveys: Current insight and future promise* p.78-95.

General Notes

Sample Selection for the Enhanced Face-to-Face Interview

In 2006, HRS initiated what is referred to as an Enhanced Face-to-Face Interview (EFTF). In addition to the core interview, the EFTF interview includes a set of physical performance tests, anthropometric measurements, blood and saliva samples, and self-administered questionnaire on psychosocial topics (the Leave-Behind).

Approximately fifty percent of households with at least one living respondent were selected for the EFTF interview across all primary sampling units (PSUs). A random one half of the 2006 sample was preselected to the EFTF interview. The other half was selected in 2008. In 2010, the first half was again interviewed, and in 2012 the second half was interviewed in person for a second time. This creates a four-year interval between biomarker collections. Similarly, new cohort households for 2010 were randomly assigned into one of these two groups.

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 EFTF interview were also asked to do so. Thus, in coupled households, both members of the couple were selected. Some respondents who were selected for the enhanced face-to-face sample were not asked to complete the physical measures or biomarkers. This group included respondents who a) needed to be interviewed by proxy, b) resided in a nursing home, or c) declined a face-to-face interview but agreed to be interviewed by telephone.

The preload variable that identifies the EFTF sample is KX090_R for 2006, LX090_R for 2008, and MX090_R for 2010 (located in the respondent preload file for each wave), for which a value of 3 indicates that the respondent was assigned to the EFTF sample for that wave. The variable EFTFASSIGN in the Cross-wave Tracker file indicates the respondents' permanent assignment for enhanced face-to-face rotation for 2006 and beyond.

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

Rationale and Key Citations

As noted above, saliva was collected on half of the HRS sample each wave starting in 2006. The cells of the cheek inside the mouth (buccal cells) can be used to provide genotypic information on individuals, and this method is well-suited to large-scale survey data collection in older populations. In 2006, saliva was collected using a mouthwash collection method. In 2008, the data collection method switched to the Oragene Collection Kit.

Using the DNA extracted from the 2006 and 2008 samples, HRS has genotyped 2.5 million single nucleotide polymorphisms (SNPs) on respondents using Illumina's Human Omni2.5-Quad (Omni2.5) BeadChip methodology. The genotyping was performed by the Center for Inherited Disease Research (CIDR) at Johns Hopkins University (<http://www.cidr.jhmi.edu/>).

The initial data product is available through the database of Genotypes and Phenotypes (dbGaP), which was established by the National Center for Biotechnology Information (NCBI) to archive and distribute data from studies investigating the interaction of genotype and phenotype. Once access to dbGaP has been granted, researchers who wish to link to HRS phenotype measures not in dbGaP may apply for access to the HRS-dbGaP Cross-Reference File by submitting a *Genetic Data Access Use Agreement*.

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 (Wallace, 2001). More information on genetics in the HRS can be found at <http://hrsonline.isr.umich.edu/gwas>.

Garcia-Closas M, et al. (2001). Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. *Cancer Epidemiology, Biomarkers, & Prevention* 10:687.

Rylander-Rudqvist T, et al. (2006). Quality and quantity of saliva DNA obtained from the self-administered Oragene method—a pilot study on the cohort of Swedish men. *Cancer Epidemiology, Biomarkers, & Prevention* 15:1742.

Zheng S, et al. (2001). Whole genome amplification increases the efficiency and validity of buccal cell genotyping in pediatric populations. *Cancer Epidemiology, Biomarkers, & Prevention* 10:697.

Wallace, R. (2001). Applying genetic study designs to social and behavioral population surveys. In Cells and surveys: Should biological measures be included in social science research? Finch, Vaupel, and Kinsella (Eds). National Academy Press, Washington, D.C.

2006 Saliva Collection

Sample

The saliva collection was intended for all of those who were available for the EFTF interview. See general sampling description (above). Special informed consent was acquired for the saliva collection process.

Consent Rate

The saliva consent rate in 2006 was 84%. The completion rate, conditional on consent, was 99%. The overall completion rate was 83%.

Measure

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.

Equipment

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 was 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 (because of the sugar alcohol in the mouthwash), 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 since the buccal (cheek) cells collected would have been removed with an insufficient quantity remaining for another collection.

2008 Saliva Collection

Sample

The saliva collection was intended for all of those who were available for the EFTF interview. See general sampling description (above). Special informed consent was acquired for the saliva collection process.

Consent Rate

The saliva consent rate in 2008 was 85%. The completion rate conditional on consent, was 99%. The overall completion rate was 84%.

Measure

A saliva sample was obtained directly using an Oragene Collection Kit. Saliva samples were sent to a central laboratory where they were processed. DNA was extracted and stored.

Equipment

Saliva Collection Kit:

- laboratory authorization form
 - plastic bag with absorbent sheet
 - Oragene collection container
- Padded mailing envelope

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 removed the Oragene saliva collection container from the plastic case.
- Interviewer handed the collection container to the respondent and instructed them to spit into the container until the bottom portion was full.
- When the respondent reached the level necessary, the interviewer put the container on a flat surface.
- A lid was placed on the collection container and securely closed. Gentle mixing for 10 seconds allowed preservative to flow from the cap to the specimen.
- 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.

Blood-based Biomarkers

Rationale and Key Citations

Cholesterol

Blood cholesterol is one of the most highly studied molecules in human biology and clinical medicine. It is 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), Low Density Lipoprotein Cholesterol (LDL-C) and Very-Low-Density-Lipoprotein Cholesterol (VLDL-C).

The DBS samples obtained in the HRS have been assayed for TC and HDL as the sample has not been asked to fast. TC (and LDC-C, which correlates very highly with TC) is positively associated with the onset of atherosclerotic cardiovascular disease, although not as strongly among older individuals. TC predicts myocardial infarction, stroke, vascular kidney disease, peripheral artery disease and many other related conditions. HDL, on the other hand, is the “good cholesterol,” and higher blood levels are associated with lower incidence of vascular conditions

generally. A given level of any blood lipid may not have the same predictive value for certain diseases in different populations for a variety of reasons, including, lack of adjustment for other risk factors, genetic and environmental differences among populations, the long term nature of disease development, and the impact of many lipid-lowering treatments on both blood levels and disease outcomes.

- Evered A. (2007). Understanding cholesterol and its role in heart disease. *NursingTimes*. 103:28-9.
- Stampfer MJ. (2006). Cardiovascular disease and Alzheimer's disease: common links. *Journal of Internal Medicine*. 260:211-23.
- Bassand JP. (2006). Managing cardiovascular risk in patients with metabolic syndrome. *Clinical Cornerstone*. 8 Suppl 1:S7-S14.
- Coryell WH. (2006). Clinical assessment of suicide risk in depressive disorder. *CNS Spectrums* 11:455-61.
- Serra M, et al. (2007). Social stress and neuroactive steroids. *European Neuropsychopharmacology* 17:1-11.
- Ashen, MD, et al. (2005). Clinical practice: Low HDL cholesterol levels. *New England Journal of Medicine* 353:1252-60.

Hemoglobin A1c

Blood glucose (sugar) levels vary in all individuals from moment to moment. A range of factors influence blood glucose, especially food consumption, and, in healthy individuals, blood glucose rises and falls throughout the day as the body responds. For those with Types I and II diabetes blood glucose is chronically elevated and must be controlled. In addition, there is substantial intra-individual variation. Even if an individual's level was measured at the same time each morning in a fasting state, the level would vary from day to day. One way to summarize blood glucose over a longer period of time is to measure glycosylated hemoglobin (HbA1c) in the blood. This is possible because 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. It is also considered a risk factor for diabetes-related conditions such as cardiovascular disease and can be used as a screening tool for diabetes and as a general indicator of anything that can potentially perturb blood glucose over time, such as altered diets, other diseases, drugs, and toxins.

- Consensus Committee. (2007). 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*. 30(9):2399-400.
- Bennett CM. (2007). HbA1c as a screening tool for detection of Type 2 diabetes: a systematic review. *Diabetes Medicine*. 24(4):333-43.

Khaw KT, et al. (2006). Glycated hemoglobin as a marker of cardiovascular risk. *Current Opinions in Lipidology* 7(6):637-43.

Reynolds, T.M., Smellie, W.S., and Twomey, P.J. (2006) Glycated haemoglobin (HbA1c) monitoring. *British Medical Journal*.16;333(7568):586-8.

C-reactive protein (CRP)

The C-reactive protein (CRP) is a blood protein that indicates levels of systemic inflammation associated with a range of acute and chronic conditions including infections, inflammatory diseases, injury, and malignancy. While elevation of this protein is part of healthy immune response, chronic elevation is associated with cardiovascular disease, hypertension, and diabetes, although it is not clear whether it plays a role in the etiology of these diseases or is a marker and a sequela of them. There is some evidence that levels of CRP are influenced by diet. Researchers who study the intersection of biological and social/psychological systems have begun to use chronic elevation of CRP as one indicator in indices of allostatic load, or the wear and tear on physiological systems associated with chronic arousal.

Clyne B, Olshaker JS (1999). "The C-reactive protein". *J Emerg Med* 17 (6): 1019–25.

Lloyd-Jones DM, Liu K, Tian L, Greenland P (June 2006). "Narrative review: assessment of C-reactive protein in risk prediction for cardiovascular disease" *Annals of Internal Medicine* 145 (1): 35–42

McEwen, B. (1998). Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York Academy of Science*, 1(840), 33-44.

Pepys MB, Hirschfield GM, Tennent GA, Gallimore JR, Kahan MC, Bellotti V, Hawkins PN, Myers RM, Smith MD, Polara A, Cobb AJ, Ley SV, Aquilina JA, Robinson CV, Sharif I, Gray GA, Sabin CA, Jenvey MC, Kolstoe SE, Thompson D, Wood SP (2006). "Targeting C-reactive protein for the treatment of cardiovascular disease". *Nature* 440 (7088): 1217–21.

Pepys, MB; Hirschfield, GM (June 2003). "C-reactive protein: a critical update". *Journal of Clinical Investigation* 111 (12): 1805–12

Cystatin C

Cystatin C is a protein produced by all human cells, used primarily as a marker of kidney function. Levels of Cystatin C appear to rise with healthy aging, but elevated levels can also be indicative of serious cardiovascular disease and impending mortality, perhaps independently of kidney functioning.

Köttgen A, Selvin E, Stevens LA, Levey AS, Van Lente F, Coresh J (2008). Serum Cystatin C in the United States: the Third National Health and Nutrition Examination Survey (NHANES III). *Am. J. Kidney Dis.* **51** (3): 385–394.

O'Hare AM, Newman AB, Katz R et al. (2005). Cystatin C and incident peripheral arterial disease events in the elderly: results from the Cardiovascular Health Study. *Arch. Intern. Med.* **165** (22): 2666–2670.

Sarnak MJ, Katz R, Fried LF et al. (2008). Cystatin C and aging success. *Arch. Intern. Med.* **168** (2): 147–153.

Stevens LA, Levey AS (2005). Chronic kidney disease in the elderly--how to assess risk. *N. Engl. J. Med.* **352** (20): 2122–2124.

Sample and Methods of Collection

Sample

The blood tests were intended for all of those who were available for the EFTF interview. See general sampling description (above). Special informed consent was acquired for the blood acquisition process.

Consent Rate

The blood spot consent rate in 2006 was 83%. The completion rate, conditional on consent, was 97%. The overall completion rate was 81%. In 2008, the blood spot consent rate in was 87%. The completion rate, conditional on consent, was 100% resulting in an overall completion rate of 87%.

Methods of Blood Determination

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 desiccant packet and then within mailing containers, and shipped to laboratories for analysis. The process is constructed so that no special temperature control is needed to preserve the values of the specimens.

Equipment

- Lab Authorization Form
- 2 Blood Collection Cards
- 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.

Laboratories Used and Assays Conducted

A number of different labs were used for the assays of HRS DBS. In 2006 and 2008 the interviewer sent the DBS samples by mail to Biosafe labs in Chicago where they were frozen. Some spots were subsequently shipped to the University of Vermont for assay of CRP and Cystatin C. Biosafe Labs went bankrupt in 2008 resulting in a switch of labs in the middle of processing the 2008 DBS. Immediately following the bankruptcy, HRS samples were retrieved from Biosafe and brought to the University of Michigan. From that point forward, interviewers mailed the samples directly to the University of Michigan, where they were sorted, frozen and shipped (in batches) to the appropriate lab for processing.

In 2006

- a. Biosafe Laboratories – assayed HbA1C, Total cholesterol and HDL cholesterol
- b. The University of Vermont assayed CRP and Cystatin C.

In 2008

- a. Two labs performed HbA1c assays - Biosafe and FlexSite (after the bankruptcy of Biosafe)
- b. Two labs assayed Total and HDL Cholesterol - Biosafe and the University of Washington (after the bankruptcy of Biosafe)
- c. The University of Vermont assayed CRP and Cystatin C

More detailed information on the assays from technical reports provided by the labs where the assays were conducted is included in Appendix A.

McDade, T. W., Williams, S., and Snodgrass, J.J. (2007). What a drop can do: Dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. *Demography*, 44(4), 899-925.

Description of HRS Blood-Based Biomarker Data

Dried Blood Spots vs. Whole Blood

Collection of dried blood spots has become increasingly common in surveys as a low cost alternative to venipuncture. There has been rapid development of assays for DBS over the past several years, although the range of assays available is still quite limited relative to whole blood. Because the resulting biomarker values based on DBS vary across assays and laboratories and may be quite different from the more conventionally used whole blood assays such as those in NHANES, and because many analysts want to make comparisons to such standard assays, we compare our results to those from NHANES. We also constructed and released a variable for each assay, which we call an NHANES equivalent value. **We recommend the NHANES equivalent assay values for analytic use.** These variables were constructed by assuming that the distribution of the DBS assays is similar to that in NHANES; we determine the value of both assays at each percentile; and then transform the DBS assays into the NHANES scale after adjusting for any between-lab differences (Details of this method are provided in Appendix B and C).

The next section begins with an overview that presents descriptive statistics on each of the five biomarkers from the HRS 2006 and 2008 data for the HRS sample with non-zero weights. We also provide some details on each assay and a comparison with NHANES values (for the 50+ NHANES sample), which are based on conventional venous blood assays.

Descriptive Data on HRS Biomarkers

The following tables provide means, standard deviations and the minimum and maximum values for each of the five biomarkers for each wave of data collection followed by the NHANES values across 2005-2008. HRS 2006 and 2008 values are weighted with the biomarker weight, which is described in the final section of this documentation report.

HRS 2006	N	Mean	Std Dev	Min	Max
Total Cholesterol (mg/dL) KTCBIOS	5797	195.44	40.85	86.00	390.00
HDL (mg/dL) KHDLBIOS	4709	57.06	14.60	21.00	133.00
HbA1c (%) KA1CBIOS	6102	5.81	0.87	4.30	13.90
CRP (ug/mL) KCRP_IMP	5818	2.11	3.48	0.02	101.64
Cystatin C (mg/L) KCYSC_IMP	5725	0.61	0.32	0.10	6.47

HRS 2008	N	Mean	Std Dev	Min	Max
Total Chol (Biosafe) (mg/dL) LTCBIO	3917	197.07	50.29	28.00	449.00
Total Chol (Univ Wash) (mg/dL) LTCUW	1718	220.48	66.42	50.61	502.75
HDL (Biosafe) (mg/dL) LHDLBIO	3461	53.78	22.65	17.95	135.00
HDL (Univ Wash) (mg/dL) LHDLUW	1718	87.43	50.29	23.65	173.50
HbA1c (Biosafe) (%) LA1CBIO	4102	5.76	0.92	3.70	13.70
HbA1c (FlexSite) (%) LA1CFLEX	1748	5.97	0.92	4.20	17.00
Cystatin C (ug/mL) LCYSC_IMP	5777	0.64	0.34	0.10	6.34
CRP (ug/mL) LCRP_IMP	5777	2.32	4.74	0.01	112.14

NHANES 2005-2008	N	Mean	Std Dev	Min	Max
Total Cholesterol (mg/dL)	4759	202.93	43.47	78.00	460.00
HDL (mg/dL)	4759	54.54	16.83	11.00	188.00
HbA1c (%)	4791	5.81	0.99	2.00	14.70
CRP (ug/mL)	4774	4.51	8.36	0.10	200.00
Cystatin C (mg/L)	3285	1.08	0.50	0.43	10.70

Comparison of values for individual markers across labs

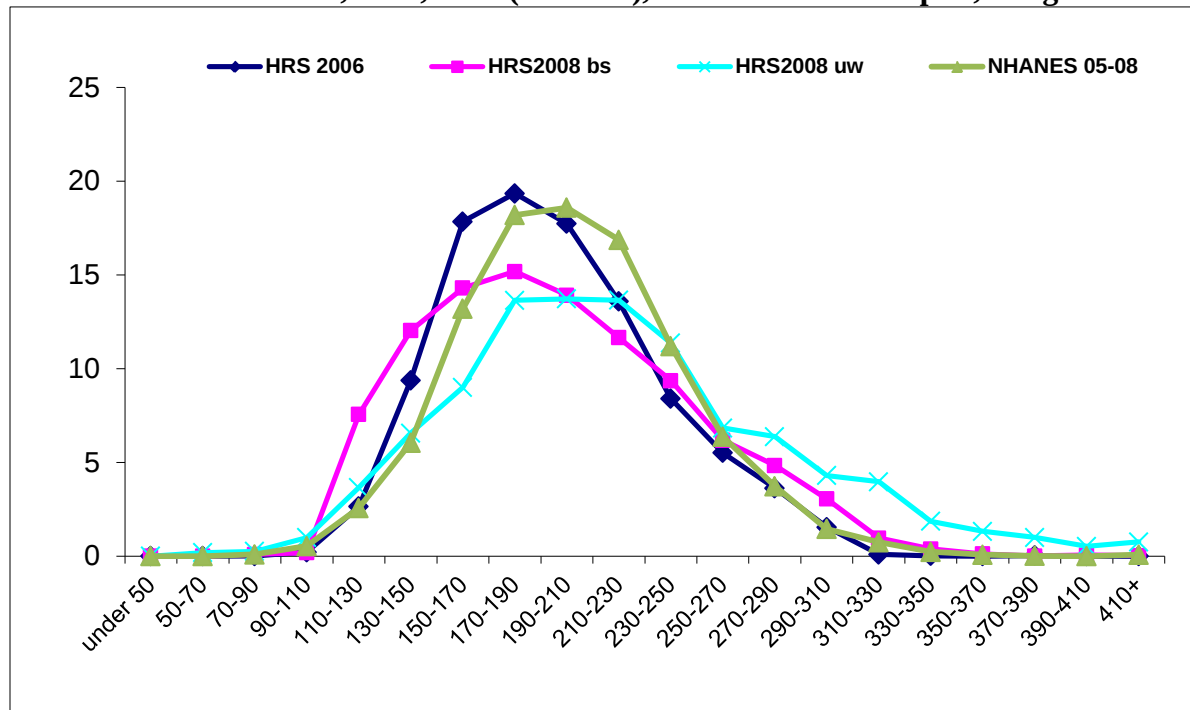
Information on the distribution for each marker from each HRS lab and for NHANES is shown at the beginning of each section. We then we present the values of the DBS assays transformed into NHANES equivalent values.

Total Cholesterol

1. HRS 2006, 2008 and NHANES 2005-2008

There are three distributions for total cholesterol from HRS samples collected in 2006 and 2008 representing the two laboratories that were used (HRS was done at the Biosafe lab in 2006 and at Biosafe and University of Washington in 2008). The University of Washington has a larger number of high values and, because of this, a higher mean and larger standard deviation. The NHANES mean is lower than the mean from the 2008 HRS sample done at the University of Washington but higher than the means for the other two HRS DBS samples. The HRS 2006 distribution looks quite similar to the NHANES distribution. With the exception of the large number of very high values in the HRS 2008 University of Washington sample, the University of Washington and the Biosafe distributions from 2008 are fairly similar. The range in the percentage of persons who would have high cholesterol (≥ 240) across these distributions is large – from 14.86 to 33.00.

Total Cholesterol in HRS, 2006, 2008 (two labs), and NHANES Samples, Weighted



Total Cholesterol (mg/dL)	N	Mean	Std Dev	%High Risk (≥ 240 mg/dL)
2006 HRS (Biosafe) KTCBIOS	5797	195.44	40.85	14.92%
2008 HRS (Biosafe) LTCBIOS	3917	197.07	50.29	20.81%
2008 HRS (Univ Wash) LTCUW	1718	220.48	66.42	33.00%
2005-08 NHANES	4759	202.93	43.47	16.00%

2. NHANES Equivalent Values

When we use percentile distributions to make NHANES or venous blood equivalent values for the HRS total cholesterol data (see Appendix B), the descriptive statistics are as follows:

Total Cholesterol (mg/dL)	N	Mean	Std Dev	%High Risk (≥ 240 mg/dL)
2006 HRS (Biosafe) KTC_ADJ	5797	202.98	43.94	18.59%
2008 HRS (Biosafe) LTCBIOS_ADJ	3917	203.10	43.50	19.80%
2008 HRS (Univ Wash) LTCUW_ADJ	1718	203.09	44.59	18.37%
2008 HRS (Combined) LTC_ADJ	5635	203.10	43.83	19.35%
2005-08 NHANES	4759	202.93	43.47	16.00%

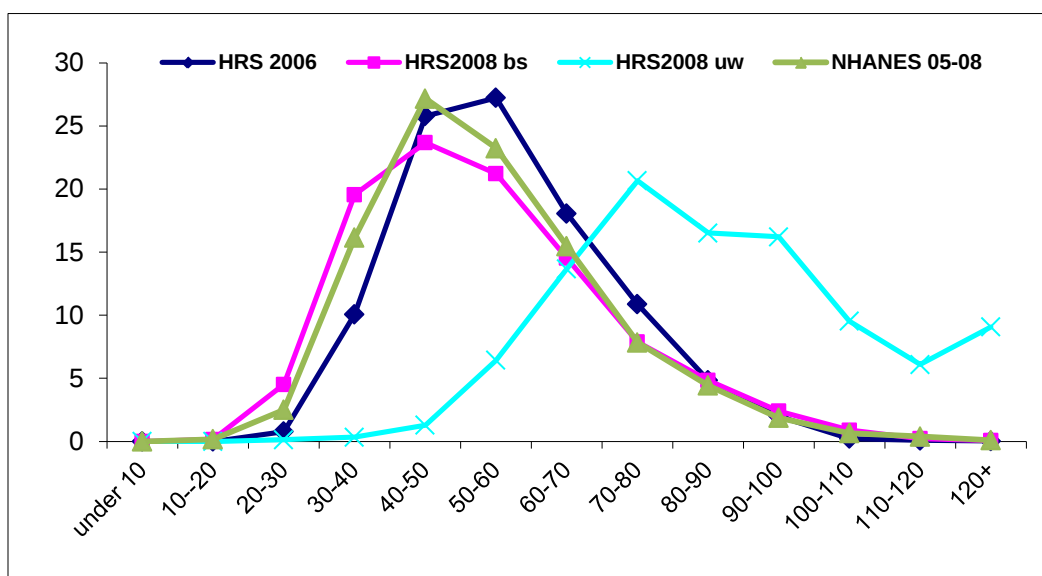
This conversion to equivalent values makes this percentage at high-risk levels in HRS similar to that in NHANES. **We recommend using the NHANES equivalent values for analysis (KTC_ADJ and LTC_ADJ).**

HDL Cholesterol

1. HRS 2006, 2008 and NHANES 2005-2008

There are three distributions for HDL cholesterol from HRS samples collected in 2006 and 2008 representing the two laboratories that were used (HRS was done at the Biosafe lab in 2006 and at Biosafe and University of Washington in 2008). The University of Washington DBS mean is substantially greater than the other means as this distribution is shifted rightward. Less than 1% are classified as high risk based on the University of Washington DBS results. The mean for NHANES is in between the means for the 2008 Biosafe and 2006 (also Biosafe) results, as is the percentage at high risk.

HDL Cholesterol in HRS, 2006, 2008 (two labs), and NHANES Samples, Weighted



HDL Cholesterol (mg/dL)	N	Mean	Std Dev	%High Risk (<40 mg/dL)
2006 HRS (Biosafe) KHDLBIOS	4709	57.06	14.60	8.94%
2008 HRS (Biosafe) LHDLBIOS	3461	53.78	22.65	21.83%
2008 HRS (Univ Wash) LHDLUW	1718	87.43	50.29	0.48%
2005-08 NHANES	4759	54.54	16.83	16.00%

2. NHANES Equivalent Values

When we construct the NHANES equivalent HDL value for the HRS sample, the descriptive statistics are as follows:

HDL Cholesterol	N	Mean	Std Dev	%High Risk (<40 mg/dL)
2006 HRS (Biosafe) KHDL_ADJ	4709	54.47	16.94	20.00%
2008 HRS (Biosafe) LHDLBIOS_ADJ	3461	54.57	16.89	19.86%
2008 HRS (Univ Wash) LHDLUW_ADJ	1718	54.63	17.23	17.72%
2008 HRS (Combined) LHDL_ADJ	5179	54.59	17.00	19.13%
2005-08 NHANES	4759	54.54	16.83	16.00%

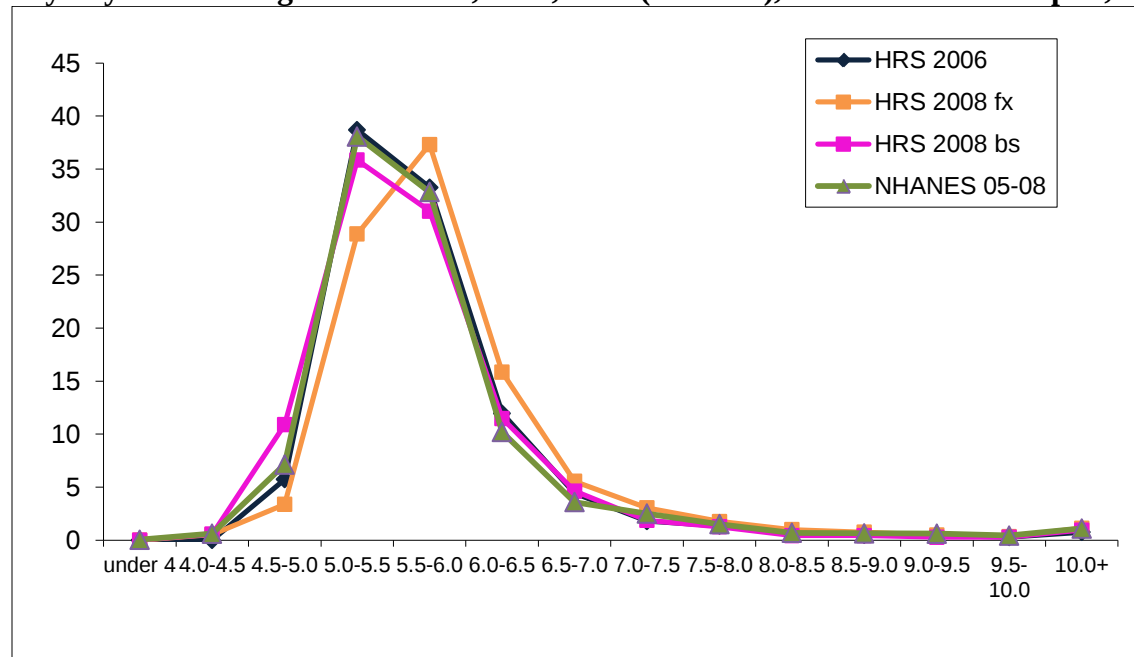
We recommend using the NHANES equivalent values for analysis (KHDL_ADJ and LHDL_ADJ).

Glycosylated Hemoglobin – HbA1c

1. HRS 2006, 2008 and NHANES 2005-2008

The distributions of glycosylated hemoglobin are quite similar across labs for DBS and for whole blood assays. The means of the NHANES value and HRS 2006 are the same. The 2008 HRS means based on the FlexSite results are slightly higher, resulting in a higher number of people who would be scored as having high levels of glycosylated hemoglobin.

Glycosylated Hemoglobin in HRS, 2006, 2008 (two labs), and NHANES Samples, Weighted



HbA1C (%)	N	Mean	Std Dev	%High HbA1c (>=6.4%)
2006 HbA1C (Biosafe) KA1CBIOS	6102	5.81	0.87	13.59%
2008 HbA1c (Biosafe) LA1CBIOS	4102	5.76	0.92	12.91%
2008 HbA1C (FlexSite) LA1CFLEX	1748	5.97	0.92	18.92%
2005-08 NHANES HbA1c	4791	5.81	0.99	13.89%

2. NHANES Equivalent Values

Descriptive information for the NHANES equivalent value of HbA1c is as follows:

HbA1C (%)	N	Mean	Std Dev	%High HbA1c ($\geq 6.4\%$)
2006 HbA1C (Biosafe) KA1C_ADJ	6102	5.81	1.03	13.59%
2008 HbA1c (Biosafe) LA1CBIOS_ADJ	4102	5.87	1.02	15.11%
2008 HbA1C (FlexSite) LA1CFELX_ADJ	1748	5.87	1.04	16.30%
2008 HbA1C (Combined) LA1C_ADJ	5850	5.87	1.03	15.48%
2005-08 NHANES HbA1c	4791	5.81	0.99	13.89%

We recommend using the NHANES equivalent values for analysis (KA1C_ADJ and LA1C_ADJ).

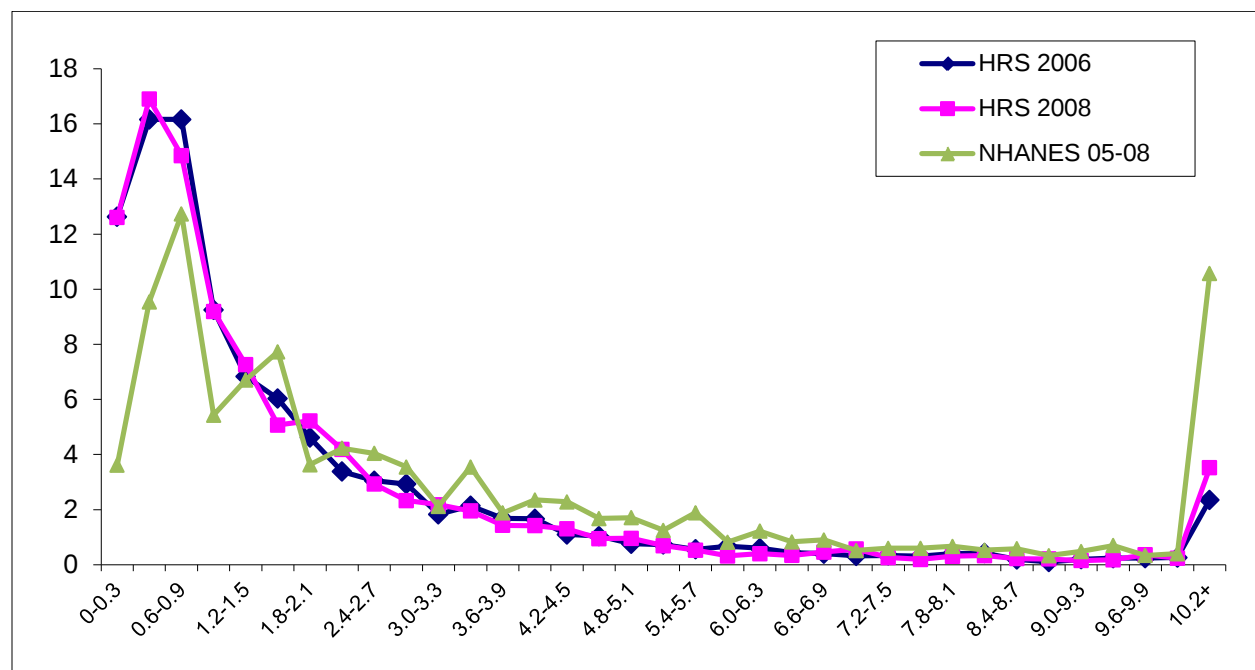
C-Reactive Protein (CRP)

1. HRS 2006, 2008 and NHANES 2005-2008

There are three distributions for CRP: HRS 2006, HRS 2008, and NHANES 2005-2008. HRS CRP for 2006 is based on DBS assays primarily done at the University of Vermont and supplemented by a small set of assays done at Biosafe. The HRS 2008 DBS were all assayed at the University of Vermont. The NHANES values for 2005-2008 are based on serum blood. Details on construction of the HRS CRP variables are included in Appendix C.

The HRS distributions for the two years are similar. The distribution of the serum values is shifted rightward. The mean and SD for the serum assay are higher than that for the DBS values.

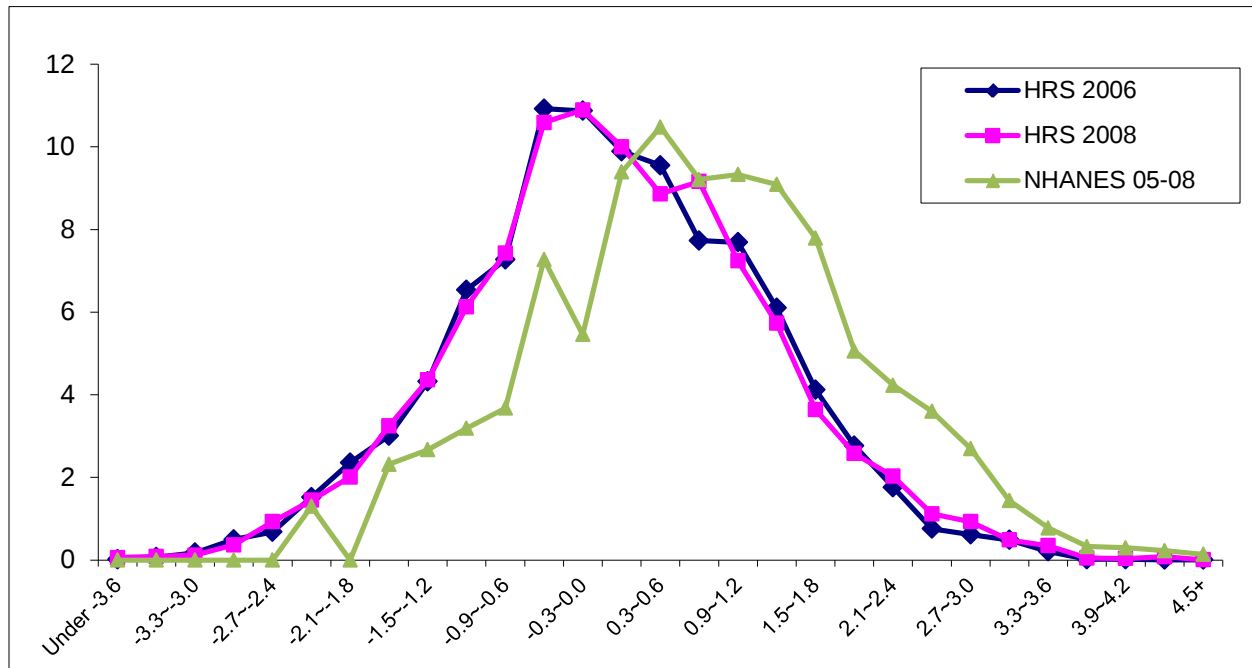
Glycosylated Hemoglobin in HRS, 2006, 2008, and NHANES Samples, Weighted



CRP (ug/mL)	N	Mean	Std Dev	%High CRP (≥ 3.0 ug/mL)
2006 HRS CRP KCRP_IMP	5818	2.11	3.48	18.98%
2008 HRS CRP LCRP_IMP	5777	2.32	4.74	19.46%
2005-08 NHANES CRP	4774	4.51	8.36	38.80%

Because of the shape of the distribution of CRP, we also provide the distribution of the logged CRP value which is often used in analysis.

Glycosylated Hemoglobin (ln) in HRS, 2006, 2008, and NHANES Samples, Weighted



2. NHANES Equivalent Values

When we use percentile distributions to make NHANES or venous blood equivalent values for the HRS data (see Appendix C), the descriptive statistics are as follows:

CRP (ug/mL)	N	Mean	Std Dev	%High CRP (≥ 3.0 ug/mL)
2006 HRS CRP KCRP_ADJ	5818	4.47	8.73	38.08%
2008 HRS CRP LCRP_ADJ	5777	4.49	9.04	38.35%
2005-08 NHANES CRP	4774	4.51	8.36	38.80%

We recommend using the NHANES equivalent values for analysis (KCRP_ADJ and LCRP_ADJ).

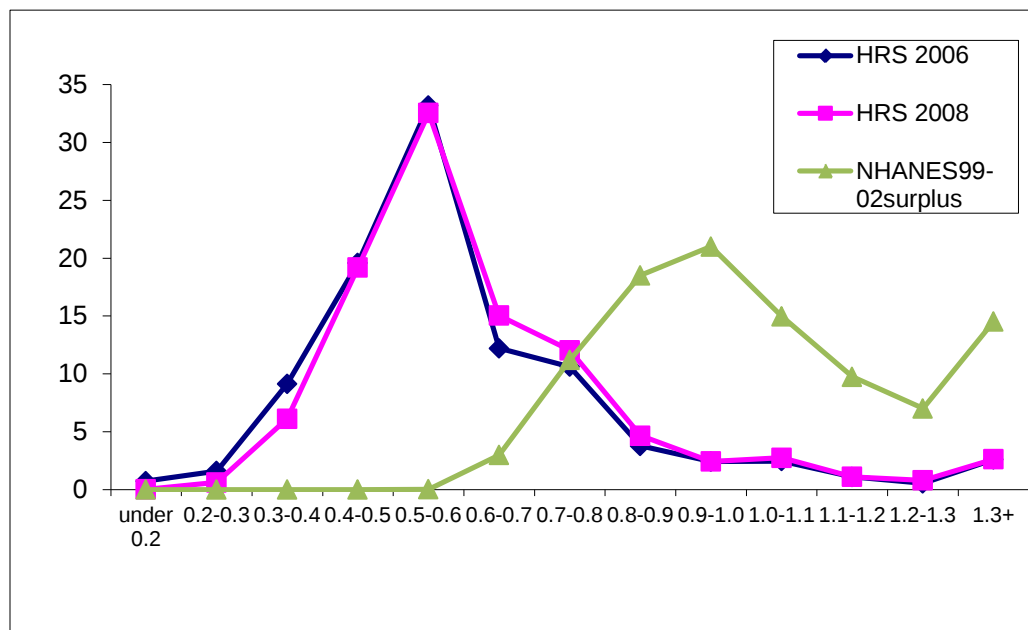
Cystatin C

1. HRS 2006, 2008 and NHANES 1999- 2002 Cystatin C

There are three distributions for Cystatin C: HRS 2006, HRS 2008, and NHANES 1999-2002. Cystatin C for HRS was assayed at the University of Vermont in both years. The NHANES values are determined using surplus serum from the years 1999-2002.

The HRS distributions are very similar across the two collections waves; the NHANES values are much higher and the distributional shape is quite different. The NHANES mean and SD are higher than the DBS values. The percentage with high Cystatin C is much higher in the NHANES sample.

Cystatin C in HRS, 2006, 2008, and NHANES Samples, Weighted



Cystatin C (mg/L)	N	Mean	Std Dev	%High Cystatin C (>1.55 (mg/L))
2006 HRS Cystatin C KCYSC_IMP	5725	0.61	0.32	1.34%
2008 HRS Cystatin C LCYSC_IMP	5777	0.64	0.34	1.51%
1999-2002 Cystatin C NHANES	3285	1.08	0.50	6.92%

2. NHANES Equivalent Values

Cystatin C (mg/L)	N	Mean	Std Dev	%High Cystatin C (>1.55 mg/L)
2006 HRS Cystatin C KCYSC_ADJ	5725	1.08	0.52	9.12%
2008 HRS Cystatin C LCYSC_ADJ	5777	1.08	0.53	8.62%
1999-2002 Cystatin C NHANES	3285	1.08	0.50	6.92%

We recommend using the NHANES equivalent values for analysis (KCYSC_ADJ and LCYSC_ADJ).

Sample Weights

Separate sample weights exist for the biomarker sub-sample in each wave. These weights, KBIOWGTR for 2006 and LBIOWGTR for 2008, can be found in the Cross-Wave Tracker File and in the biomarker data file for each collection wave.

The biomarker sample weight is the product of the HRS core sampling weight and a non-response adjustment factor. The HRS sampling weight from the concurrent interview was used as the base weight. The nonresponse adjustment factor was obtained from a propensity model predicting the probability of completing the biomarker portion of the eFTF interview among those selected and eligible to participate. The propensity model was estimated by logistic regression and weighted by the base weight. Predictor variables included age, sex, race/ethnicity, education, coupleness, self-rated health, number of physical limitations and report of a chronic health condition (i.e., diabetes, use of diabetes medications, hypertension, heart conditions, myocardial infarction, angina, congestive heart failure or stroke). Predictor variables were taken from the current interview. The inverse of the fitted probability of completion formed the non-response adjustment factor. Finally, the weights were post-stratified to closely match the HRS sample composition by age, gender, and race.

Appendix A – Laboratory Technical Reports

Biosafe

Biosafe provided their own card for collection of the blood used for total and HDL cholesterol and HbA1c. The spot used for HbA1c had additional pre-treatment.

Biosafe reported that the within-run precision of their DBS **total cholesterol assay** ranged from 3.3% to 3.8% as the mean value of cholesterol went from 153 mg/dL to 263 mg/dL. The between-run precision ranged from 3.7% to 3.2% as total cholesterol went from 153 mg/dL to 263 mg/dL. Biosafe reported that total cholesterol was stable for more than 28 days at room temperature and also after enduring temperature change for 21 days. Biosafe reported a strong correlation between serum blood values and DBS values in their lab (.965), indicating high accuracy. The equation linking DBS cholesterol and serum cholesterol that was provided by Biosafe was $DBS = 0.997 \text{ serum} + 1.18$. The total error of determination provided by Biosafe which combines accuracy and precision is 10.0% when total cholesterol is 153; 10.3% when total cholesterol is 196; and 9.8% when total cholesterol is 263.

Biosafe reported that the within-run precision of their DBS **HDL cholesterol assay** ranged from 3.0% to 3.3% as the mean value of HDL cholesterol went from 42.6 mg/dL to 79.5 mg/dL. The between-run precision was 6.4% at 42.6 mg/dL and 4.3% at 79.5 mg/dL. Biosafe reported that HDL cholesterol was stable for more than 28 days at room temperature and also after enduring temperature change for 21 days. Biosafe reported a strong correlation between serum blood values and DBS values in their lab (.95) indicating high accuracy. The equation linking DBS HDL cholesterol and serum HDL cholesterol that was provided by Biosafe was $DBS = 0.9405 \text{ serum} + 2.3001$. The total error of determination provided by Biosafe which combines accuracy and precision is 13.9% when HDL cholesterol is 42.6 and 13.8% when HDL cholesterol is 79.5.

FlexSite

FlexSite developed the A1c AT HOME™ Kit for monitoring diabetes control. The assay for **HbA1c** is performed using the Roche Unimate immunoassay and the Cobas Integra Analyzer, which is calibrated using a synthetic HbA1c standard. The Roche Unimate method is an immunoassay that combines a latex-enhanced competitive turbidimetric immunoassay for determining HbA1c with a colorimetric assessment of total Hb. The Cobas Integra analyzer, which is used in the Roche Unimate method, was certified by the National Glycohemoglobin Standardization Program (NGSP) in 1997. The Bio-Rad Variant HPLC method, utilizing ion exchange HPLC to separate HbA1c is also NGSP certified. A1c AT HOME™ was cleared by the Food and Drug Administration (FDA) for at-home use and over-the-counter sale in 1997.

FlexSite evaluated their assay with a sample of patients who collected blood samples at home using the kit and mailed the samples to the lab. The time from the home spotting to the arrival of the spots at FlexSite ranged from 1 to 5 days with the average being 2.5 days. The time lapse between the arrival and assay of spots and the collection of the original whole blood sample ranged from 2 to 14 days with the average being 5.7 days. The results indicated that the A1c AT HOME™ Test results based on DBS are highly correlated with the standard Roche Unimate

immunoassay on whole blood. The correlation between the home spots and the whole blood tests was .956 and the equation linking the two was DBS A1c = .9826 whole blood + .369.

The A1c at home kit was also compared to the Dimat ion-exchange/HPLC method which has been used in a program to standardize and certify measurement of HbA1c. In this comparison the $r = .98$ and the equation is:

$$\text{DBS A1c} = .9995 \text{ Diamet} + .0003.$$

The precision estimates for the A1c AT HOME™ are provided below.

	<u>Normal</u>	<u>Elevated</u>
Within Run CV	0.88%	1.71%
Between Run CV	1.31%	1.90%
Total (20 day) CV	1.46%	2.54%

FlexSite has determined that the DBS samples are stable to typical mailing conditions and to conditions of extreme desert heat.

University of Washington

Cholesterol Assay

The DBS assay for **total cholesterol (TC)** done at the University of Washington is a fluorimetric assay. A punch from a DBS card is eluted with a buffer solution. The elution solution is incubated with assay reagent containing cholesterol ester hydrolase, cholesterol oxidase, peroxidase, and a fluorogen. The cholesterol ester hydrolase catalyzes the conversion of cholesterol esters to cholesterol, and this and de novo cholesterol is oxidized by cholesterol oxidase, producing hydrogen peroxide (H_2O_2) as a byproduct. In the presence of the peroxidase, the H_2O_2 reacts with the non-fluorescent fluorogen to produce a fluorescent fluorophore. The TC concentration is directly proportional to the fluorescent intensity of the solution; fluorescence is measured spectrophotometrically. A standard curve is constructed by plotting the fluorescence values of the standards against the known TC concentrations. Using the standard curve, the fluorescence values of the QC samples and patient samples are read as TC concentrations. Acceptability of the assay is determined by comparing the TC concentrations of the QC samples with their established values.

DBS TC assay calibrators were constructed from high TC concentration pooled human plasma (University of Washington Department of Laboratory Medicine, Seattle, WA; UW Lab Med) serially diluted with 7% bovine serum albumin in phosphate buffered saline (BSA/PBS; Sigma Aldrich, St. Louis, MO) to the desired final TC concentration. Two DBS QC samples were constructed from a separate pool of human plasma, either undiluted (high TC concentration QC sample) or diluted with BSA/PBS (low TC concentration QC sample). Each calibrator and QC sample solution was mixed with a constant volume of washed human erythrocytes (UW Lab Med), pipetted in 75 μl aliquots onto No. 903 filter paper (Whatman, Piscataway, NJ) and dried for 4hr at room temperature (RT) (23°C). The final TC concentration of each calibrator and QC sample solution was determined by analysis on a UniCel DxC 800 Synchron Clinical System (Beckman Coulter, Miami, FL). DBS calibrators, QC samples and study samples were sealed in

Ziploc bags with desiccant packs and stored at -70°C until processing. Immediately prior to processing, DBS were warmed to RT and then a single 3.2mm (1/8in) diameter punch was punched from each DBS into a microtiter plate well (Greiner Bio-One, Monroe, North Carolina). The samples were either immediately assayed or the microtiter plates were firmly sealed and stored at -70°C pending assaying.

Immediately prior to assaying, microtiter plates were warmed to RT. A TC elution buffer comprised of 400µl ddH₂O with 0.1% Triton X-100 (Sigma) was added to each microtiter plate well. The plate was sealed and gently shaken for 1hr on a microplate shaker (Delfia Plateshake, PerkinElmer, Waltham, MA) to elute TC from each punch. 20 µl of eluent was transferred from each well of the elution plate to an assay microtiter plate. This was followed by addition of 100 µl of TC assay reagent comprised of 70% Cholesterol Chromogen (Synermed, Westfield, IN), 29% Cholesterol Enzyme (Synermed) and 1% Cayman ADHP (10-acetyl-3,7-dihydroxyphenoxazine; Cayman Chemical, Ann Arbor, MI). The assay plate was gently shaken for 30sec and then incubated at 37°C for 30min. The fluorescence intensity (RFU) of each well was read at 530/25nm excitation and 590/35nm emission on a microtiter plate reader (Synergy HT, BioTek, Winooski, VT). A linear regression calibration curve, constructed by plotting the assigned concentrations of the calibrators against the recorded fluorescence values, was used to convert the blank-subtracted RFU value of each sample into a DBS TC concentration (Gen5 Software, BioTek).

The TC assay lower limit of detection is 27mg/dl, within-assay imprecision (CV) is 4.0% and between-assay imprecision is 4.7%. The TC concentrations of 105 DBS samples analyzed by the DBS assay correlated with the TC concentrations of paired plasma samples (Pearson R = 0.93) and were linearly related (DBS assay TC value = 17.141 + plasma-equivalent TC value X 1.582). Correction for hematocrit did not improve the agreement between the serum and DBS TC values. The DBS assay for **HDL cholesterol (HDL)** done at the University of Washington is a fluorometric assay. A punch from a DBS card is eluted with deionized water. The elution solution is combined with a reagent containing anti-human β-lipoprotein antibody (to bind non-HDL lipoproteins into nonreactive complexes) and a fluoren. A second reagent, containing cholesterol ester hydrolase, cholesterol oxidase and peroxidase, is then added. The cholesterol ester hydrolase catalyzes the conversion of HDL into cholesterol, which is in turn oxidized by cholesterol oxidase and produces hydrogen peroxide (H₂O₂) as a byproduct. In the presence of the peroxidase, H₂O₂ reacts with the non-fluorescent fluorogen to produce a fluorescent fluorophore. The HDL concentration is directly proportional to the fluorescent intensity of the solution; fluorescence is measured spectrophotometrically. A standard curve is constructed by plotting the fluorescence values of the standards against the known HDL concentrations. Using the standard curve, the fluorescence values of the QC samples and respondent samples are read as HDL concentrations. Acceptability of the assay is determined by comparing the HDL concentrations of the QC samples with their established values.

DBS HDL assay calibrators were constructed from high HDL concentration pooled human plasma (University of Washington Department of Laboratory Medicine, Seattle, WA; UW Lab Med) serially diluted with 7% bovine serum albumin in phosphate buffered saline (BSA/PBS; Sigma Aldrich, St. Louis, MO) to the desired final HDL concentration. Two DBS QC samples were constructed from a separate pool of human plasma, either undiluted (high HDL concentration QC sample) or diluted with BSA/PBS (low HDL concentration QC sample). Each

calibrator and QC sample solution was mixed with a constant volume of washed human erythrocytes (UW Lab Med), pipetted in 75µl aliquots onto No. 903 filter paper (Whatman, Piscataway, NJ) and dried for 4hr at RT (23°C). The final HDL concentration of each calibrator and QC sample solution was determined by analysis on a UniCel DxC 800 Synchron Clinical System (Beckman Coulter, Miami, FL). DBS calibrators, QC samples and study samples were sealed in Ziploc bags with desiccant packs and stored at -70°C until processing. Immediately prior to processing, DBS were warmed to RT and then a single 3.2mm (1/8in) diameter punch was punched from each DBS into a microtiter plate well (Greiner Bio-One, Monroe, North Carolina). The samples were either immediately assayed or the microtiter plates were firmly sealed and stored at -70°C pending assaying.

Immediately prior to assaying, microtiter plates were warmed to RT. 400µl diH₂O HDL elution buffer was added to each microtiter plate well. The plate was sealed and gently shaken for 1hr on a microplate shaker (Delfia Plateshake, PerkinElmer, Waltham, MA) to elute HDL from each punch. 40µl of eluent was transferred from each well of the elution plate to an assay microtiter plate. This was followed by addition of 75µl of HDL assay reagent 1 comprised of 99% EZ HDL Cholesterol Reagent 1 (Trinity Biotech, St Louis, MO) and 1% Cayman ADHP (10-acetyl-3,7-dihydroxyphenoxazine; Cayman Chemical, Ann Arbor, MI). The assay plate was gently shaken for 30sec and then incubated at 37°C for 25min. At the conclusion of this incubation, 25µl of HDL assay reagent 2 (EZ HDL Cholesterol Reagent 2; Trinity Biotech) was added, the assay plate shaken for 30sec and then incubated at 37°C for 30min. The fluorescence intensity (RFU) of each well was read at 530/25nm excitation and 590/35nm emission on a microtiter plate reader (Synergy HT, BioTek, Winooski, VT). A linear regression calibration curve, constructed by plotting the assigned concentrations of the calibrators against the recorded fluorescence values, was used to convert the blank-subtracted RFU value of each sample into a DBS HDL concentration (Gen5 Software, BioTek).

The HDL assay lower limit of detection is 15mg/dl, within-assay imprecision (CV) is 5.3% and between-assay imprecision is 9.9%. The HDL concentrations of 104 DBS samples analyzed by the DBS assay correlated with the HDL concentrations of paired plasma samples (Pearson R = 0.88) and were linearly related (DBS assay HDL value = 32.941 + plasma-equivalent HDL value X 0.965). Correction for hematocrit did not improve the agreement between the serum and DBS HDL values.

High-Sensitivity C-Reactive Protein (hsCRP) Assay

The **CRP** Assay is a sandwich ELISA. A punch from a dried blood spot (DBS) card containing either a CRP assay standard, a quality control (QC) sample or a patient sample is eluted in a buffer solution. The elution solution is transferred to an ELISA microtiter plate. The bottom face of each well of the ELISA plate is pre-coated with an anti-CRP monoclonal antibody (mAb) that binds CRP in the elution solution (solid phase immobilization). A conjugate solution containing anti-CRP Ab coupled to peroxidase (enzyme-linked antibody) is then added to each well resulting in CRP molecules being sandwiched between the solid phase and enzyme-linked antibodies. A tetramethylbenzidine (TMB) and hydrogen peroxide (H₂O₂) solution is added; H₂O₂, cleaved by the peroxidase, reacts with TMB and causes the solution to develop a blue color. The CRP concentration is directly proportional to the absorbance of the solution; absorbance is measured spectrophotometrically. A standard curve is constructed by plotting the

absorbance values of the standards against the known CRP concentrations. Using the standard curve, the absorbance values of the QC samples and patient samples are read as CRP concentrations. Acceptability of the assay is determined by comparing the CRP concentrations of the QC samples with their established values.

DBS CRP assay calibrators were constructed from pooled human plasma with a negligible CRP concentration (negligible CRP plasma; University of Washington Department of Laboratory Medicine, Seattle, WA; UW Lab Med) spiked with CRP concentrate (Cell Sciences, Canton, MA) and serially diluted with negligible CRP plasma to the desired final CRP concentrations. Three DBS QC samples were constructed from a separate pool of human plasma, either undiluted (high CRP concentration QC sample) or diluted with negligible CRP plasma (medium CRP concentration QC sample and low CRP concentration QC sample). Each calibrator and QC sample solution was mixed with a constant volume of washed human erythrocytes (UW Lab Med), pipetted in 75µl aliquots onto No. 903 filter paper (Whatman, Piscataway, NJ) and dried for 4hr at RT (23°C). The final CRP concentration of each calibrator and QC sample solution was determined by analysis on a UniCel DxC 800 Synchron Clinical System (Beckman Coulter, Miami, FL). DBS calibrators, QC samples and study samples were sealed in Ziploc bags with desiccant packs and stored at -70°C until processing. Immediately prior to processing, DBS were warmed to RT and a single 3.2mm (1/8in) diameter punch was punched from each DBS into a microtiter elution plate well (Greiner Bio-One, Monroe, North Carolina). The samples were either immediately assayed or the microtiter plates were firmly sealed and stored at -70°C pending assaying.

Immediately prior to assaying, microtiter plates were warmed to RT. 200µl CRP elution buffer (hsCRP Sample Diluent; Percipio, Inc, Manhattan Beach, CA) was added to each plate well. The plate was sealed and gently shaken for 1hr on a Delfia Plateshake microplate shaker (PerkinElmer, Waltham, MA) to elute CRP. 20µl of eluent was transferred from each well of the elution plate to an ELISA microtiter plate followed by addition of 100µl of CRP Enzyme Conjugate Reagent (Percipio). The ELISA plate was gently shaken at RT for 45min and then washed 5X with di/ddH₂O. 100µl of TMB Reagent (Percipio) was added to each well and the plate placed on a microtiter plate reader (Synergy HT, BioTek, Winooski, VT). The absorbance of each well (OD) was read at 370nm excitation after the OD of the high standard on the plate reached 1.5 (approximately 20min). A 5-parameter weighted calibration curve, constructed by plotting the assigned concentrations of the calibrators against the recorded absorbance values, was used to convert the blank-subtracted OD value of each sample into a DBS CRP concentration (Gen 5 Software, BioTek).

The CRP assay lower limit of detection is 0.035mg/L, within-assay imprecision (CV) is 8.1% and between-assay imprecision is 11.0%. The CRP concentrations of 87 DBS samples analyzed by the DBS assay correlated with the CRP concentrations of paired plasma samples (Pearson R = 0.99) and were linearly related (DBS assay CRP value = 0.370 + plasma-equivalent CRP value X 1.077).

University of Vermont

C-Reactive protein (CRP) and Cystatin C Assays

CRP is measured using the BNII nephelometer (Siemens, Inc., Deerfield, IL) utilizing a particle enhanced immunonephelometric assay using a monoclonal antibody to CRP. Blood spot specimens were assayed at a final dilution of 1:83.3. The lowest detectable value is 0.63 ug/mL. Upper limit is 40 ug/mL at this dilution. If the sample had sufficient volume, they were rediluted and rerun which allows for a lower limit of 0.03 ug/mL and an upper limit of 800 ug/mL. Almost half the sample still had values in the nondetectable range and they were rerun using an Elisa assay (see below).

Expected values for CRP in normal, healthy individuals are ≤ 3 ug/mL.

Cystatin C (Cys C) is measured using the BNII nephelometer (Siemens, Inc., Deerfield, IL) utilizing a particle enhanced immunonephelometric assay using a monoclonal antibody to Cystatin C. Blood spot specimens are assayed at a final dilution of 1:83.3. The lowest detectable value is 0.22 mg/L in part 1 (2006 set) and 0.20 mg/L in part 2 (2008 set).

Expected values for Cystatin C in normal, healthy individuals are approximately 0.53 – 0.95 mg/L.

The Siemens BNII is a fully automated instrument used in the analysis of plasma and serum protein. The analyzer uses nephelometry to determine the quantities of a given analyte. Essentially, latex beads are attached to a specific molecule (the analyte), each latex bead has a specific light scattering molecule attached. Light is passed through the sample, and based on the amount of light scattering sensed by the detector, the quantities of a specific analyte are determined.

Dried Blood Spot Eluates for the BNII:

Using a 3.0mm hole punch, 2 complete blood spot disks (equivalent to approximately 3 uLs serum) were punched and added into one 1.5ml skirted cryovial. 250ul of BNII dilution buffer was then added to this cryovial resulting in elution of 3ul of serum producing a 1:83.3 dilution.

CRP in sample with non-detectable results by nephelometry were rerun using a quantitative sandwich Elisa (R&D Systems # DCRP00, Minneapolis, MN) which uses a monoclonal antibody specific for CRP. Blood spot specimens were assayed by Elisa at a final dilution of 1:50 using 2 punches of the blood spot card, equivalent to approximately 3 uLs serum, added to 147 uLs assay diluent. The lowest detectable value by this method is approximately 0.01 ug/mL with a high detection of 2.5 ug/mL.

Among those with low values based on the BNII nephelometer method, in the 2006 samples there are 280 missing results due to insufficient sample for rerun by Elisa. The 2008 samples have 123 missing results due to insufficient sample for rerun by Elisa. In the final variable

constructed from both the BNII and the Elisa runs, 13% of the cases have missing values in 2006 and 4.3% in 2008.

University of Vermont Assay Summary:

HRS Blood Spots					Inter-assay CVs	
	Assays	Method	Run Dates	Reported	Serum Controls	DBS Controls
Set 1 (2006)	CRP	Seimens BNII Nephelometry	Jan 2009 to June 2009	6/24/2009	4.36% to 7.96%	10.99%
	Cystatin C	Seimens BNII Nephelometry	Jan 2009 to June 2009	6/24/2009	3.39% to 8.35%	10.93%
	CRP Rerun	R&D Systems Elisa	Feb 2011 to June 2011	6/30/2011	7.42% to 9.95%	7.61% and 10.82%
Set 2 (2008)	CRP	Seimens BNII Nephelometry	Dec 2009 to April 2010	5/4/2010	3.82% to 5.84%	9.22% and 3.03%
	Cystatin C	Seimens BNII Nephelometry	Dec 2009 to April 2010	5/4/2010	2.20% to 6.22%	9.93% and 4.68%
	CRP Rerun	R&D Systems Elisa	Oct 2010 to Jan 2011	2/1/2011	9.41% to 15.49%	13.0% and 10.87%

Appendix B

Constructing NHANES Equivalent Values

The DBS values differ from the NHANES values where assays are analyzed from samples of whole blood. Both NHANES and HRS are population-based studies and they are intended to represent the non-institutional U.S. population when sampling weights are used. For some purposes it may be advantageous to adjust the HRS DBS values to levels consistent with NHANES, exploiting the fact that the population distributions should be the same if there are no differences in lab procedures. This is one way to make the level of the HRS data more similar to that based on conventional assays while still preserving the variability in the sample.

Approach

We first calculate the values of the assays corresponding to (weighted) 100 percentiles in HRS and in NHANES. For HRS we use the biomarker weights (KBIOWGTR and LBIOWGTR). [To facilitate construction of percentiles when values are discrete and have many individuals scored at the same value, we first add a very small random number to each observed value, create the (weighted) percentiles based on the altered values, and then take the mean of the actual assay values at each percentile]. For NHANES, we pool the 2005-06 and 2007-08 samples for assays other than Cystatin C. For Cystatin C, the NHANES comparison data are from 1999-2002.

We then have 100 percentiles for HRS and 100 percentiles for NHANES. (Because of the highly skewed distribution of CRP, we work with log values). We then regress the HRS value on the NHANES value to create an equation that can be used to convert HRS values into NHANES equivalent values.

Appendix C

Construction of HRS CRP and Cystatin C for 2006 and 2008

1. CRP

Vermont assayed many of the DBS samples two times for CRP because the first assay had a level of detection that was higher than the level of CRP in many samples. A second Elisa assay with a much lower level of detection was done on these samples when possible (identified with the variable KCRP_FLAG and LCRP_FLAG). The released CRP variables from Vermont (KCRPVT and LCRPVT) use the Vermont DBS value if it is available - either from the first assay if the value was high enough for detection or the Elisa assay done on cases with low values from the first run. In 2006, this approach was supplemented with material from Biosafe for small number of assays.

- a. 2006 – There are 286 cases with both a CRP value from Vermont and Biosafe. The correlation between these is .98 with means and SD at Vermont (mean 2.82 (SD 3.49)) and Biosafe (4.71 (SD 6.20)). After adjusting the Biosafe value to correspond to the Vermont value using the ratio of means, the correlation between the two remains .98 (SD 3.71). This allows us to use Biosafe values adjusted to the Vermont scale for 131 cases with data from Biosafe, but not Vermont.
- b. This leaves 222 cases with no CRP value because they were too low for detection in the first assay; they have no Elisa value and no Biosafe value. These 222 cases are assigned a value using a hotdeck approach. The hotdeck consists of 1898 cases that did not have a detectible value in the first assay but have either an Elisa value from Vermont or a value from Biosafe. When this is done the mean for the imputed values is .409 and the range is .035 to 1.163. These imputed cases are identified using the variable KCRP_IMPFLAG.
- c. For 2008, 125 cases were imputed using the hotdeck method – mean .454 (.024 to 1.164). These imputed cases are identified using the variable LCRP_IMPFLAG.

2. Cystatin C

For cases below the detectible limit (.22 in 2006 and .20 in 2008) values of .1 are assigned. In 2006 there is only 1 case and in 2008 there are 47 cases. These cases are flagged with an imputation flag (KCYSC_IMPFLAG and LCYSC_IMPFLAG)