HRS | HEALTH AND RETIREMENT STUDY
A Longitudinal Study of Health, Retirement, and Aging
Sponsored by the National Institute on Aging

Documentation of Blood-Based Biomarkers in the 2014 Health and Retirement Study

Documentation Report

Eileen Crimmins, University of Michigan
Jessica Faul, University of Michigan
Jung Ki Kim, University of Southern California
David Weir, University of Michigan

Survey Research Center
Institute for Social Research
University of Michigan
Ann Arbor, Michigan

December 2017

Funding

The Health and Retirement Study is funded by a grant from the National Institute on Aging (U01 AG009740) with supplemental support from the Social Security Administration. HRS is conducted by the University of Michigan.

Suggested Citation

Crimmins, E., Faul, J., Kim, J & Weir, D. (2017). Documentation of Blood-Based Biomarkers in the 2014 Health and Retirement Study. University of Michigan.
https://hrs.isr.umich.edu/publications/biblio/9387

Introduction

This document describes the HRS blood-based biomarker data collected and assayed from 2014. More detailed descriptions of the procedures for collection of the data and assay of the blood-based markers as well as the genetic markers are available on the HRS website.

Documentation for data from previous years are available in the following reports:

Crimmins, E. M., Faul, J. D., Kim, J. K., Guyer, H. Langa, K. M., Ofstedal, M. B., Sonnega, A., Wallace, R. B., & Weir, David R. (2013). *Documentation of biomarkers in the 2006 and 2008 Health and Retirement Study.* Retrieved from the Health and Retirement Study website: http://hrsonline.isr.umich.edu/modules/meta/bio2008/desc/Biomarker2006and2008.pdf

Crimmins, E. M., Faul, J. D., & Weir, David R. (2015). *Documentation of biomarkers in the 2010 and 2012 Health and Retirement Study.* Retrieved from the Health and Retirement Study website: http://hrsonline.isr.umich.edu/modules/meta/bio2012/desc/Biomarker2010and2012.pdf

Blood-Based Biomarkers in the HRS

HRS began to collect blood-based biomarkers on half the sample in 2006, the other half of the sample provided biomarker data in 2008. The first group was asked for blood samples again in 2010 and 2014; the second group gave repeat samples in 2012 and 2016.

From 2006 through 2012, the dried blood spot (DBS) samples were assayed for 5 biomarkers. In 2014 a sixth was added, IL-6 which is a cytokine indicator of inflammation. The biomarkers available now in the 2014 release include the following:

a. Total cholesterol (TC) an indicator of lipid levels
b. High Density Lipoprotein cholesterol (HDL), an indicator 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

The IL-6 data will be released when we have data from VBS available to make an equivalent value.

Laboratories

A series of labs have been used over the years to assay HRS DBS. However, in 2014, as in 2012, the University of Washington performed all of the assays.

University of Washington Department of Medicine Dried Blood Spot Laboratory

Immunology Division, Department of Laboratory Medicine
Director: Mark H. Wener, MD, wener@u.washington.edu
Project Director: Alan Potter, Ph.D., apotter@uw.edu

Procedures

Sample. The blood tests were intended for all of those who were available for the EFTF interview. Special informed consent was acquired for the blood acquisition process.

Consent Rate. The blood spot consent rate in 2014 was 90.7%. The completion rate, conditional on consent, was 99%. The overall completion rate was 90.3%.

Shipping. In 2014, interviewers mailed the DBS cards directly to the University of Michigan, where they were sorted, frozen and shipped (in batches) to the appropriate lab for processing.

Description of 2014 Blood-Based Biomarker Data

Assay Values from Dried Blood Spots vs. 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, and because many analysts want to make comparisons to such standard assays, we compare our results to those from a similarly aged nationally representative sample with conventional assays, the National Health and Nutrition Examination Survey (NHANES). We have 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.

Comparison of the HRS DBS values and those from venous blood assays is described in detail in the previously listed HRS biomarker reports, and in the following:

Crimmins, E. M., Kim, J. K., McCreath, H., & Seeman, T. (2013). *Results from the Health and Retirement Study Biomarker Validation Project.* Retrieved from the Health and Retirement Study website: http://hrsonline.isr.umich.edu/sitedocs/genetics/HRSBiomarkerValidation.pdf

Crimmins, E. M., Kim, J. K., McCreath, H., Faul, J. D., Weir, D. R., & Seeman, T. (2014). Validation of blood-based assays using dried blood spots for use in large population studies. *Biodemography and Social Biography, 60*(1), 38-48. doi:10.1080/19485565.2014.901885

These sources make it clear that different lab assays and procedures result in different assay values. As mentioned above and described more below, the HRS solution to the problem of different assays is to produce an equivalent value using the distribution in a study which uses conventional assays.

Constructing NHANES Equivalent Values

The equivalent values make the assay levels for the HRS data based on DBS similar to the level in NHANES where values are based on conventional assays while the variability in the HRS sample is preserved. Because the weighted NHANES and HRS samples are both population-based studies intended to represent the non-institutional U.S. population, we adjust the HRS DBS values for total cholesterol (TC), high density lipoprotein (HDL), glycosylated hemoglobin (HbA1c), C-reactive protein (CRP) and cystatin C to levels consistent with NHANES, exploiting the fact that the population distributions should be the same if there are no differences in lab procedures.

Briefly, the 2014 HRS data are compared to NHANES data from 2011-2014 for total cholesterol, HDL and Hba1c; 2009-2010 for CRP; and 1999-2002 for cystatin C. We describe why this is and how to examine longitudinal data. For the HRS 2006 and 2008 assays the pooled sample for NHANES 2005-2006 and NHANES 2007-2008 provided the reference for 4 analytes: TC, HDL, HbA1c, and CRP. For HRS 2010 and 2012, the NHANES samples for NHANES 2009-2010 and 2011-2012 were the reference for TC, HDL, and HbA1c. For 2014 HRS TC, HDL and HbA1c, NHANES data from 2011-2014 were the basis for equivalent values. For HRS 2010-2012 CRP, only NHANES 2009-2010 was the reference as later CRP data were not available. No more NHANES data have become available for CRP, so HRS 2014 equivalent values are also based on NHANES 2009-2010. Because cystatin C has not been regularly done in NHANES, HRS 2006, 2008, 2010, 2012, and 2014 all use the same NHANES 1999-2002 for cystatin C to construct equivalent values.

This means that average differences in HRS samples from 2006 to 2010 to 2014 and from 2008 to 2012 will reflect differences, or change over time, in NHANES values of TC, HDL, and HbA1c. There is no time change reflected in the cystatin C measure. For CRP, there is no time change reflected in the HRS 2010-12 and 2014 values.

Our approach is to first calculate the values of the assays corresponding to (weighted) 100 percentiles in HRS and in NHANES. For HRS we use the biomarker weights (OBIOWGTR). 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 2011-12 and 2013-14 samples for TC, HDL, and HbA1c. For cystatin C, the NHANES comparison data are from 1999-2002 and are the same data used for the other years for HRS assays. For 2014 CRP we use the 2009-10 NHANES data. We then have 100 percentiles for HRS and 100 percentiles for NHANES. Because of the highly skewed distribution of CRP, we log the values before we create the percentiles and run the regression on 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.

The following equations were applied to create NHANES equivalent variables of biomarkers:

OA1C_ADJ = -2.444194 + OA1CUW * 1.511696

OTC_ADJ = 1.405374 + OTCUW * 0.7086605
OHDL_ADJ = -0.2485097 + OHDLUW * 0.8134292
OCYSC_ADJ = 0.0331803 + OCYSCUW * 1.305697
OCRP_ADJ = exp(-0.6279805 + 1.259465*log(OCRPUW))

Description of HRS Blood-Based Biomarker data

This section begins with an overview that presents descriptive statistics on each of the 6 biomarkers from the HRS 2014 for the 50+ HRS sample with nonzero weights. We also provide some comparison with NHANES values for the 50+ sample which are based on conventional venous blood assays. We also provide descriptive statistics for the HRS NHANES equivalent values. In subsequent sections, we provide details on each assay.

The following tables provide means, standard deviations and the minimum and maximum values for each of the five biomarkers in HRS 2014 followed by the NHANES comparison data. The HRS data are weighted the wave appropriate biomarker weight.

HRS 2014

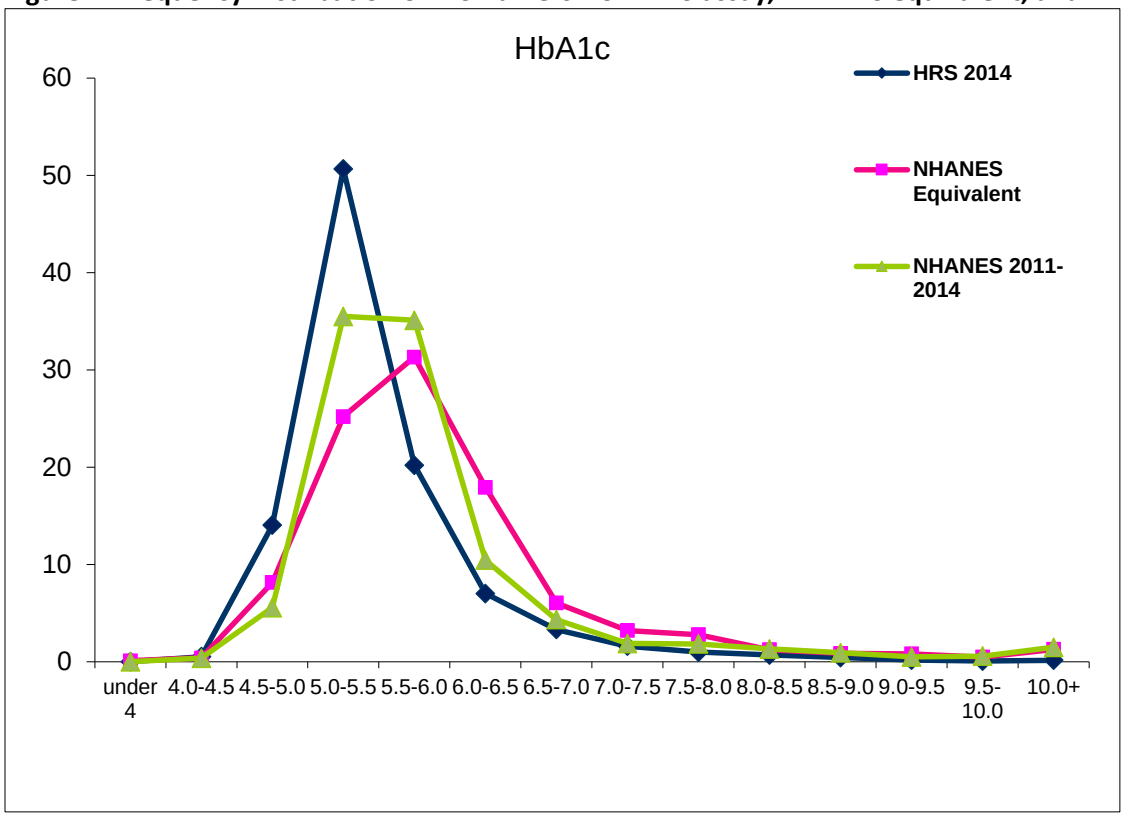
HRS 2014 assay	N	Mean	SD	Min	Max
HbA1c (%) OA1CUW	6776	5.56	0.71	4.00	12.10
Total Cholesterol (mg/dL) OTCUW	6698	275.61	60.09	146.00	535.00
HDL (mg/dL) OHDLUW	6237	67.65	20.49	6.32	267.56
Cystatin C (mg/L) OCYSCUW	6770	0.84	0.40	0.12	8.74
CRP (ug/mL) OCRPUW	6749	3.88	5.98	0.06	112.67
Logged CRP	6749	0.69	1.16	-2.81	4.72
NHANES					
HbA1c (%)	5022	5.91	1.09	3.50	17.80
Total Cholesterol (mg/dL)	4921	196.67	43.93	75.00	525.00
HDL (mg/dL)	4921	54.77	17.34	16.00	175.00
Cystatin C(1999-2002) (mg/L)	3285	1.08	0.50	0.43	10.70
CRP (2009-2010) (ug/mL)	2388	3.53	6.56	0.10	113.20
Logged CRP (2009-10)	2388	0.23	1.48	-2.30	4.73
HRS 2014 NHANES Equivalent Value					
HbA1c (%) OA1C_ADJ	6776	5.96	1.07	3.60	15.85
Total Cholesterol (mg/DL) OTC_ADJ	6698	196.72	42.58	104.87	380.54
HDL (mg/dL) OHDL_ADJ	6237	54.78	16.67	4.89	217.39
Cystatin C (mg/L) OCYSC_ADJ	6770	1.13	0.52	0.19	11.44
CRP (ug/mL) OCRP_ADJ	6749	3.63	8.21	0.02	204.86
Logged CRP	6749	0.24	1.47	-4.17	5.32

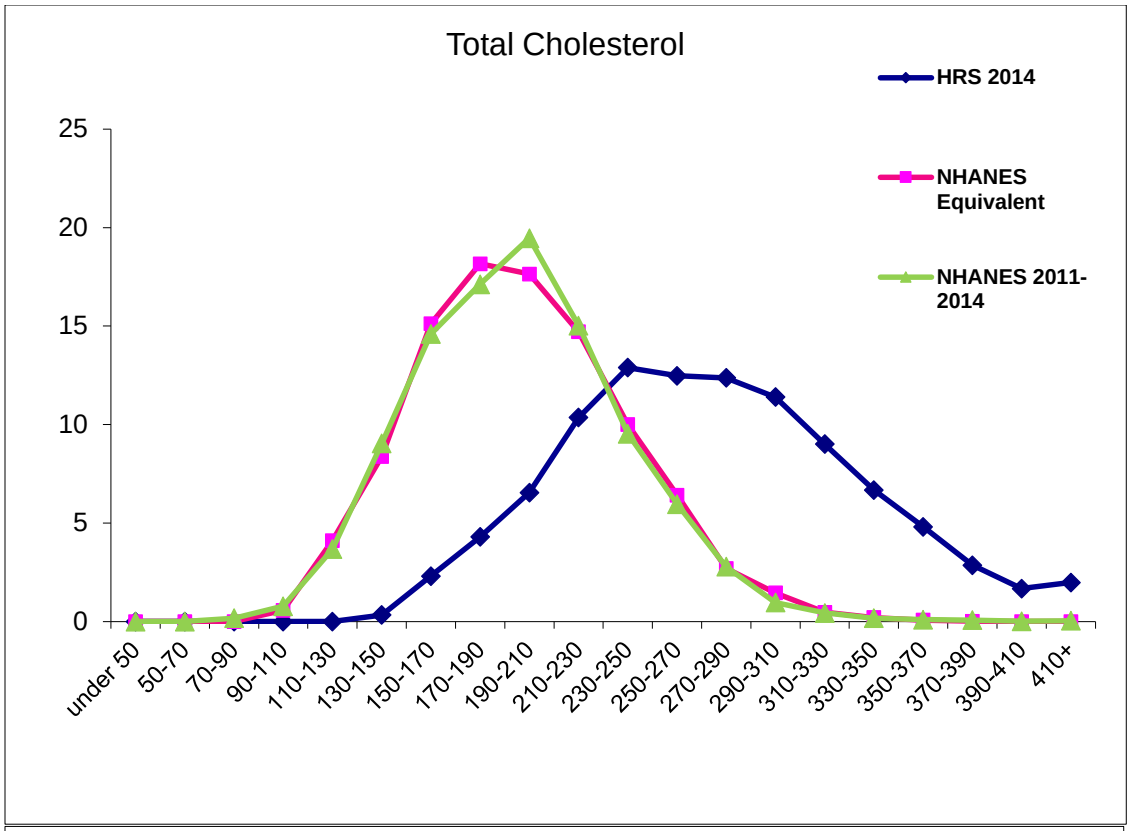
Sample Weights

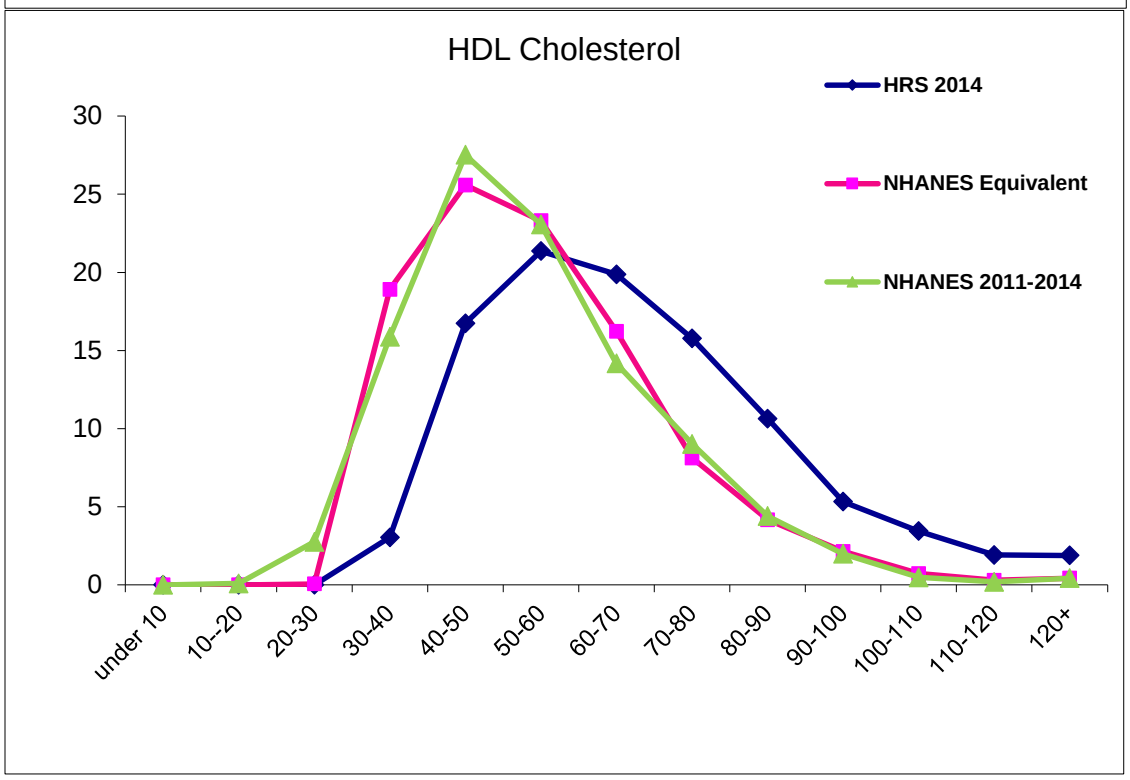
Separate sample weights exist for the biomarker sub-sample in each wave. These weights, OBIOWGTR for 2014, can be found in the Cross-Wave Tracker File and in the biomarker data file for each collection wave. The biomarker sample weight is the product of the HRS core sampling weight and a non-response 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

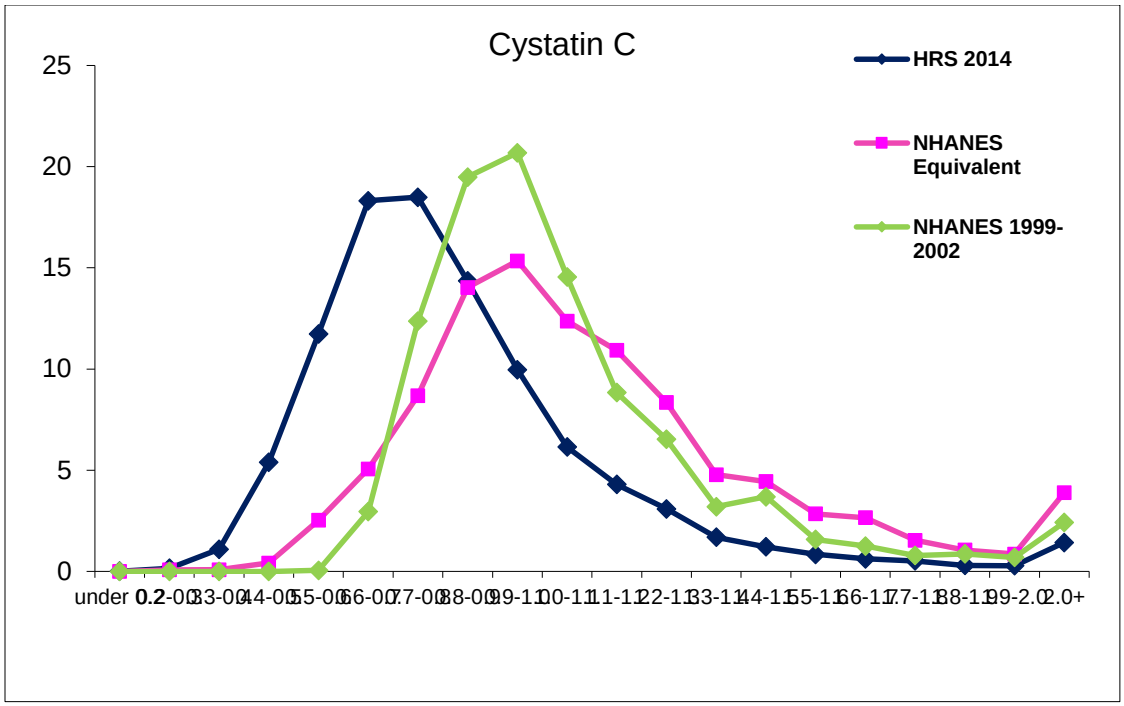
regression and weighted by the base weight. Predictor variables included age, sex, race/ethnicity, education, coupleness, self-rated health, number of physical limitations and report of a chronic health condition (i.e., diabetes, use of diabetes medications, hypertension, heart conditions, myocardial 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.

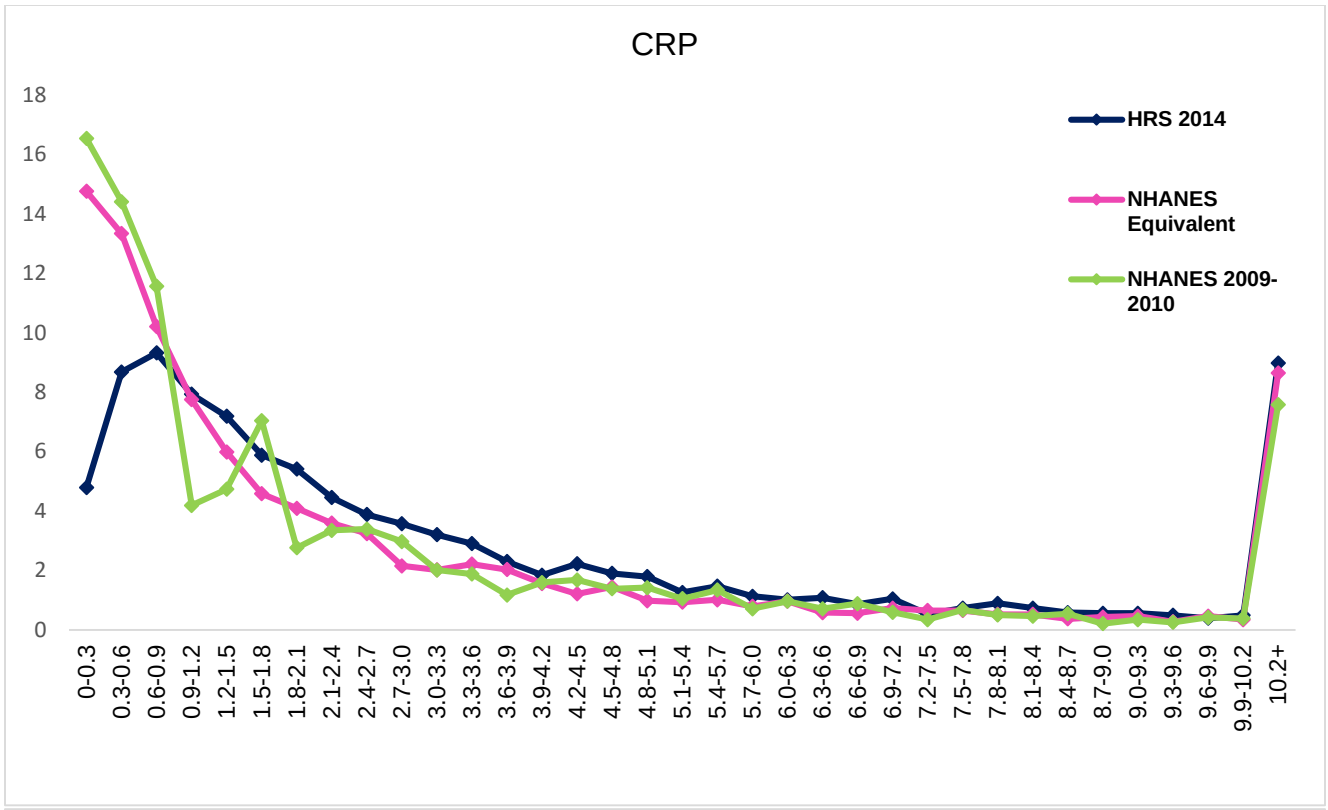
Figure 1. Frequency Distribution of Biomarkers from HRS assay, NHANES equivalent, and NHANES values

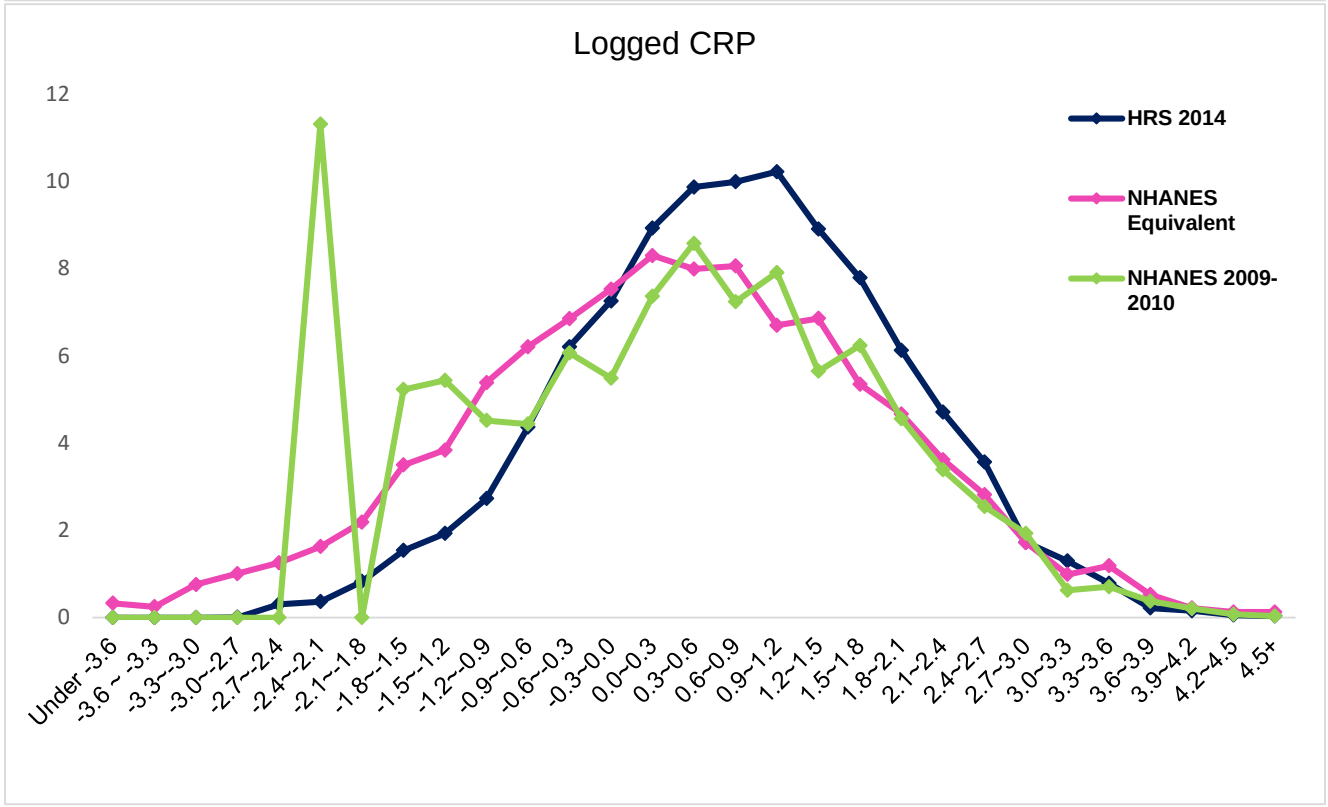



Total Cholesterol
HRS 2014
NHANES Equivalent
NHANES 2011-2014
25
20
15
10
5
0
under 50
50-70
70-90
90-110
110-130
130-150
150-170
170-190
190-210
210-230
230-250
250-270
270-290
290-310
310-330
330-350
350-370
370-390
390-410
410+


HDL Cholesterol
HRS 2014
NHANES Equivalent
NHANES 2011-2014
30
25
20
15
10
5
0
under 10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
80-90
90-100
100-110
110-120
120+


Cystatin C
HRS 2014
NHANES Equivalent
NHANES 1999-2002
25
20
15
10
5
0
under 0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1.0
1.0-1.1
1.1-1.2
1.2-1.3
1.3-1.4
1.4-1.5
1.5-1.6
1.6-1.7
1.7-1.8
1.8-1.9
1.9-2.0
2.0+


CRP
18
16
14
12
10
8
6
4
2
0
HRS 2014
NHANES Equivalent
NHANES 2009-2010
0-0.3
0.3-0.6
0.6-0.9
0.9-1.2
1.2-1.5
1.5-1.8
1.8-2.1
2.1-2.4
2.4-2.7
2.7-3.0
3.0-3.3
3.3-3.6
3.6-3.9
3.9-4.2
4.2-4.5
4.5-4.8
4.8-5.1
5.1-5.4
5.4-5.7
5.7-6.0
6.0-6.3
6.3-6.6
6.6-6.9
6.9-7.2
7.2-7.5
7.5-7.8
7.8-8.1
8.1-8.4
8.4-8.7
8.7-9.0
9.0-9.3
9.3-9.6
9.6-9.9
9.9-10.2
10.2+


Logged CRP
12
10
8
6
4
2
0
HRS 2014
NHANES Equivalent
NHANES 2009-2010
Under -3.6
-3.6~-3.3
-3.3~-3.0
-3.0~-2.7
-2.7~-2.4
-2.4~-2.1
-2.1~-1.8
-1.8~-1.5
-1.5~-1.2
-1.2~-0.9
-0.9~-0.6
-0.6~-0.3
-0.3~0.0
0.0~0.3
0.3~0.6
0.6~0.9
0.9~1.2
1.2~1.5
1.5~1.8
1.8~2.1
2.1~2.4
2.4~2.7
2.7~3.0
3.0~3.3
3.3~3.6
3.6~3.9
3.9~4.2
4.2~4.5
4.5+

Description of Assays: This material is derived from technical reports provided Alan Potter who directs the HRS Laboratory work at the University of Washington

Dried blood spot (DBS) quality control (QC) samples, DBS assay calibrators and DBS study samples are sealed in Ziploc bags with desiccant packs and stored in -70°c freezers (Revco Ultima Plus, Thermo Fisher Scientific, Pittsburg, PA) at UW Lab Med (University of Washington Department of Laboratory Medicine, Seattle, WA). Prior to processing, DBS are warmed to room temperature (RT). A single 3.2mm (1/8in) diameter disc is punched from each DBS sample for each assay (four discs for the IL-6 assay) into a deep-96 well microtiter plate well (Greiner Bio-One, Monroe, North Carolina) by a BSD700 Semi-Automated Dried Sample Puncher (BSD Robotics, Brisbane, QLD, Australia). Microtiter plates are then either immediately assayed or are sealed (CapMat, Greiner Bio-One) and stored at -70°c. Frozen microtiter plates are warmed to RT prior to assaying.

The DBS assay for **Total Cholesterol** (TC) run at UW Lab Med is a fluorimetric assay. A buffer solution (UW Lab Med) is added to each microtiter well containing a DBS disc and the plate is then sealed and gently shaken on a Delfia Plateshake microplate shaker (PerkinElmer, Waltham, MA) for 1hr at RT to elute TC. An aliquot of the eluate is transferred to an assay microtiter plate followed by addition of TC assay reagent (UW Lab Med) containing cholesterol ester hydrolase, cholesterol oxidase, peroxidase, and a fluorogen. The assay plate is gently shaken for 30sec and then incubated at 37°c for 30min. 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 (RFU) of the solution. RFU is read at 530/25nm excitation and 590/35nm emission on a Synergy HT microtiter plate reader (BioTek, Winooski, VT). A linear regression calibration curve is constructed by plotting the recorded RFU values of the calibrators against the assigned TC concentrations (Gen5 Software, BioTek). The calibration curve is used to convert the RFU values of the QC samples and the study samples into DBS TC direct concentrations. Acceptability of the assay is determined by comparing the TC concentrations of the QC samples with the established values.

DBS TC assay calibrators were constructed from high TC concentration pooled human plasma (UW Lab Med) serially diluted with bovine serum albumin in phosphate buffered saline (BSA/PBS; Sigma Aldrich, St. Louis, MO) to the desired final concentration. Two DBS QC samples (UW Lab Med) 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 solution and QC sample solution was mixed with a constant volume of washed human erythrocytes (UW Lab Med), pipetted in aliquots onto Whatman No. 903 filter paper (GE Healthcare Bio-Sciences, Pittsburgh, PA) and dried. The TC concentration of each calibrator solution and QC sample solution was determined by analysis on an Olympus AU680 Chemistry System (Beckman, Brea, CA).

The TC assay lower limit of detection (LLOD) is 27mg/dl, within-assay imprecision (CV) is 3.7% and between-assay CV is 7.0%. The TC concentrations of 72 DBS samples analyzed by the DBS assay correlated with the TC concentrations of paired plasma samples

(Pearson R = 0.95) and were linearly related (DBS direct TC concentration = 40 + plasma-equivalent TC concentration X 1.404).

The DBS assay for **HDL cholesterol** (HDL-c) run at UW Lab Med is a fluorimetric assay. A buffer solution (UW Lab Med) is added to each microtiter well containing a DBS disc and the plate is then sealed and gently shaken on a Delfia Plateshake microplate shaker for 1hr at RT (PerkinElmer) to elute HDL-c. An aliquot of the eluate is transferred to an assay microtiter plate followed by addition of HDL-c Assay Reagent 1 (UW Lab Med) containing anti-human β-lipoprotein antibody to bind non-HDL lipoproteins into nonreactive complexes and a flurogen. The assay plate is gently shaken for 30sec and then incubated at 37°c for 25min. Assay Reagent 2 containing cholesterol ester hydrolase, cholesterol oxidase and peroxidase (UW Lab Med) is added and the plate is shaken for 30sec and then incubated at 37°c for 30min. Cholesterol ester hydrolase catalyzes the conversion of HDL-c into cholesterol, which is in turn oxidized by cholesterol oxidase and produces H_2O_2 as a byproduct. In the presence of the peroxidase, H_2O_2 reacts with the non-fluorescent fluorogen to produce a fluorescent fluorophore. The HDL-c concentration is directly proportional to the RFU of the solution; fluorescence in each well is read at 530/25nm excitation and 590/35nm emission on a Synergy HT microtiter plate reader (BioTek). A linear regression calibration curve is constructed by plotting the recorded RFU values of the calibrators against the assigned HDL-c concentrations (Gen5 Software, BioTek). The standard curve is used to convert the RFU values of the QC samples and the study samples into DBS HDL-c direct concentrations. Acceptability of the assay is determined by comparing the HDL-c concentrations of the QC samples with the established values.

DBS HDL-c assay calibrators were constructed from high HDL-c concentration pooled human plasma (UW Lab Med) serially diluted with 7% bovine serum albumin in BSA/PBS (Sigma) to the desired final concentration. Two DBS QC samples were constructed from a separate pool of human plasma either undiluted (high HDL-c concentration QC sample) or diluted with BSA/PBS (low HDL-c 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 Whatman No. 903 filter paper (GE Healthcare) and dried for 4hr at RT. The HDL-c concentration of each calibrator solution and QC sample solution was determined by analysis on a Olympus AU680 Chemistry System (Beckman).

The HDL-c assay LLOD is 15mg/dl, within-assay CV is 3.0% and between-assay CV is 7.0%. The HDL-c concentrations of 104 DBS samples analyzed by the DBS assay correlated with the HDL-c concentrations of paired plasma samples (Pearson R = 0.88) and were linearly related (DBS direct HDL-c concentration = 23 + plasma-equivalent HDL-c concentration X 1.380).

The DBS **hemoglobin A1c** (HbA1c) Assay run at UW Lab Med uses an automated ion-exchange high-performance liquid chromatography (IE-HPLC) system to measure the percentage of glycosylated hemoglobin (%HbA1c). HbA1c buffer (Bio-Rad Hemoglobin A1c Program Wash/Diluent Reagent, Bio-Rad Laboratories, Hercules, CA) is added to each microtiter plate well and the plate is then sealed and vigorously shaken for 1hr on a Delfia Plateshake microplate shaker (PerkinElmer) to reliquefy the dried blood. The reliquefied blood is transferred to an HbA1c sample vial containing Wash/Diluent Reagent (Bio-Rad), gently shaken for 30sec and then analyzed on a Variant II Hemoglobin Testing System (Bio-

Rad). The Variant II performs a chromatographic separation of glycosylated HbA1c on a cation exchange cartridge by applying a programmed buffer gradient of increasing ionic strength to separate hemoglobins based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell of a filter photometer where absorbance is measured at 415nm. The chromatogram curves are integrated to determine the HbA1c and total HbA areas (Variant II Clinical Data Management Software, Bio-Rad). %HbA1c is determined from the HbA1c:total HbA ratio adjusted by the slope and intercept of the calibration curve (Bio-Rad)

DBS QC samples were constructed by pipetting 75µl aliquots of blood with known %HbA1c values onto Whatman No. 903 filter paper (GE Healthcare) and drying for 4hr at RT (UW Lab Med). Acceptability of the assay is determined by comparing the %HbA1c concentrations of two QC samples (Bio-Rad Lyphochek Bilevel Diabetes Control) and DBS QC samples at the beginning, middle, and end of each assay run against established values. Acceptability of the analysis of each sample is determined by examining the chromatogram for proper form, absence of interfering peaks, acceptable total area, and %HbA1c value within the analytical measurement range (AMR).

The HbA1c assay AMR is 3.1% to 18.5% per established limits (Bio-Rad). The within-assay CV is 2.5% and between-assay CV is 2.9%. The %HbA1c values of 283 DBS samples analyzed by the DBS assay correlated with the %HbA1c values of DBS-matched liquid blood samples (Pearson R = 0.98) and were linearly related (blood %HbA1c value = -2.245 + DBS direct %HbA1c value X 1.378).

The DBS **cystatin C** (cysC) Assay run by UW Lab Med is a sandwich ELISA. cysC Dilution Buffer (BioVendor, Candler, NC) is added to each microtiter well containing a DBS disc and the plate is then sealed and vigorously shaken on a Delfia Plateshake microplate shaker (PerkinElmer) for 1hr at RT, held overnight at 4°c, and then vigorously shaken for 1hr at RT to elute cysC. An aliquot of the eluate is transferred to an ELISA microtiter plate (BioVendor) pre-coated with an antibody (Ab) that recognizes and binds cysC (solid phase immobilization). The plate is gently shaken for 60min at RT in the dark and then washed 5X with cysC Wash Solution (BioVendor). cysC Conjugate Solution (BioVendor) containing anti-cysC Ab coupled to peroxidase (enzyme-linked antibody) is then added to each well resulting in cysC being sandwiched between the solid phase and enzyme-linked Ab. The plate is gently shaken for 30min at RT in the dark and then washed 5X with cysC Wash Solution. Tetramethylbenzidine (TMB) Reagent containing H_2O_2 (Biovendor) is added and the plate is gently shaken for 12min at RT in the dark; H_2O_2, cleaved by the peroxidase, reacts with TMB and causes the solution to develop color. The reaction is stopped by adding Stop Solution (BioVendor) and gently shaking the plate for 30min at RT in the dark. The cysC concentration is directly proportional to the absorbance (OD) of the solution; OD at 450nm excitation is measured on a Synergy HT microtiter plate reader (BioTek). A 5-parameter calibration curve, constructed by plotting the OD values of the calibrators against the assigned cysC concentrations, is used to convert the OD value of each sample into a DBS direct cysC concentration (Gen 5 Software, BioTek). Acceptability of the assay is determined by comparing the cysC concentrations of the QC samples with the established values.

DBS cysC assay calibrators were constructed from whole blood obtained from a single donor (Puget Sound Blood Center (PSBC), Seattle, WA). High cysC concentration plasma

was created by pooling high cysC concentration plasma (UW Lab Med) and spiking with recombinant human cysC concentrate (Sigma). cysC calibrators were created by centrifuging aliquots of the whole blood and replacing volumes of plasma with equal volumes of high cysC concentration plasma; increasing volumes of plasma were replaced to create increasing cysC concentration calibrators. Low cysC concentration calibrators were created by replacing volumes of plasma with equal volumes of 5% Human Albumin Solution (ZLB Behring; Berne, Switzerland); increasing volumes of plasma were replaced to create decreasing cysC concentration calibrators. DBS QC samples were constructed from whole blood (PSBC) obtained from a donor with a high cysC concentration (high cysC QC sample) and from whole blood obtained from a donor with a low cysC concentration (low cysC QC sample). 75µl aliquots of each calibrator and QC sample whole blood was pipetted onto Whatman No. 903 filter paper (GE Healthcare) and dried for 4hr at RT. The final cysC concentration of each calibrator solution and QC sample solution was determined by analyses on a Seimens Dade-Behring BN II Nephelometer (Deerfield, IL).

The cysC assay LLOD is 0.025µg/ml, within-assay CV is 3.6% and between-assay CV is 5.7%. The cysC concentrations of 91 DBS samples analyzed in duplicate by the DBS assay correlated with the cysC concentrations of DBS-matched plasma samples measured in duplicate by nephelometry (Pearson R = 0.96) and were linearly related (plasma cysC concentration = 0.256 + DBS direct cysC concentration X 0.999).

The DBS high-sensitivity **C-reactive protein** (hsCRP) Assay run at UW Lab Med is a sandwich ELISA. hsCRP Sample Diluent (Percipio, Inc, Manhattan Beach, CA) is added to each microtiter well containing a DBS disc and the plate is then sealed and gently shaken for 1hr on a Delfia Plateshake microplate shaker (PerkinElmer) to elute CRP. An aliquot of the eluate is transferred to an ELISA microtiter plate (Percipio) pre-coated with an anti-CRP monoclonal antibody (mAb) that recognizes and binds CRP (solid phase immobilization). CRP Enzyme Conjugate Reagent (Percipio) containing anti-CRP Ab coupled to peroxidase (enzyme-linked antibody) is then added to each well resulting in CRP being sandwiched between the solid phase and enzyme-linked antibodies. The plate is gently shaken at RT for 45min and then washed 5X with di/ddH2O. TMB Reagent containing H_2O_2 (Percipio) is added; H_2O_2, cleaved by the peroxidase, reacts with TMB and causes the solution to develop a blue color. The plate is placed onto a Synergy HT microtiter plate reader (BioTek) and the OD of each well is read at 370nm excitation after the OD of the high standard on the plate reaches 1.5 (approximately 20min). The CRP concentration is directly proportional to the OD of the solution. A 5-parameter calibration curve is constructed by plotting the OD values of the calibrators against the assigned CRP concentrations (Gen 5 Software, BioTek). The calibration curve is used to convert the OD value of each sample into a DBS direct CRP concentration. Acceptability of the assay is determined by comparing the CRP concentrations of the QC samples with the established values.

DBS CRP assay calibrators were constructed from pooled human plasma with a negligible CRP concentration (UW Lab Med) spiked with CRP concentrate (Cell Sciences, Canton, MA) and serially diluted with negligible CRP plasma to the desired final 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 Whatman No. 903 filter paper (GE Healthcare) and dried for 4hr at RT. 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).

The CRP assay LLOD 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 direct CRP concentration = 0.370 + plasma-equivalent CRP concentration X 1.077).