Documentation of DBS Blood-Based Biomarkers in the 2016 Health and Retirement Study

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Introduction

This document describes the HRS DBS based biomarker data collected and assayed from 2016. This is the last time DBS based markers will be collected in HRS. DBS have been collected from approximately half the sample in each year beginning in 2006. Detailed descriptions of the procedures for collection and assay of DBS based data as well as VBS blood-based markers beginning in 2016 are available on the HRS website.

Documentation for the 2006 and 2008 data are provided in "**Documentation of Biomarkers in the 2006 and 2008 Health and Retirement Study**." 2013. Eileen Crimmins, Jessica Faul, Jung Ki Kim, Heidi Guyer, Kenneth Langa, Mary Beth Ofstedal, Amanda Sonnega, Robert Wallace and David Weir.
http://hrsonline.isr.umich.edu/modules/meta/bio2008/desc/Biomarker2006and2008.pdf

Documentation for the 2010 and 2012 data are provided in "**Documentation of Biomarkers in the 2010 and 2012 Health and Retirement Study**." 2015. Eileen Crimmins, Jessica Faul, Jung Ki Kim and David Weir.
http://hrsonline.isr.umich.edu/modules/meta/bio2012/desc/Biomarker2010and2012.pdf

Documentation for the 2014 data are provided in "**Documentation of Biomarkers in the 2014 Health and Retirement Study**." 2017. Eileen Crimmins, Jessica Faul, Jung Ki Kim and David Weir.
https://hrs.isr.umich.edu/sites/default/files/biblio/Biomarker%202014_Dec2017.pdf

Additional documentation for the 2014 data are provided in "**HEALTH AND RETIREMENT STUDY Sensitive Health Data Blood-Based Biomarkers 2014 Health and Retirement Study Data Description and Usage**." Version 1.0, December 2017.
https://hrsdata.isr.umich.edu/sites/default/files/documentation/data-descriptions/Biomarker2014DD_0.pdf

Blood-Based Biomarkers in the HRS

HRS began to collect DBS blood-based biomarkers on half the sample in 2006, the other half of the sample provided DBS 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 6th was added, IL-6 which is a cytokine indicator of inflammation. The biomarkers available now in the 2014 release and for 2016 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 for both years will be released shortly as we now have VBS data to use to make VBS equivalent values.

Laboratories

A series of labs have been used over the years to assay HRS DBS. However, in 2012, 2014 and 2016 the University of Washington did all assays.

University of Washington Department of Medicine Dried Blood Spot Laboratory
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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 2016 was 86.7%. The completion rate, conditional on consent, was 98.9%. The overall completion rate was 85.7%.

Collection. DBS samples were collected halfway through the interviews (Section I) by trained interviewers by filling up to ten circles with blood droplets across two Whatman blood spot cards. DBS cards were placed in a specially-designed cardboard box allowing airflow on all sides for a minimum of two hours of drying time prior to shipment. The loaded cardboard boxes were placed in placed in foil pouches with desiccant.

Shipping. In 2016, interviewers mailed the DBS cards directly to the Department of Laboratory Medicine at the University of Washington in Seattle for assay. On receipt, the lab staff in Washington coded the quality and characteristics of the DBS cards. DBS cards were stored at -70°C prior to and after analysis.

Assays

DBS Glycosylated Hemoglobin (HbA1c) Assay. The dried blood spot (DBS) glycosylated hemoglobin (HbA1c) assay was performed using a Bio-Rad Laboratories Variant II High Pressure Liquid Chromatography (HPLC) System (Hercules, CA) optimized to accommodate the limited microliter volume available from a DBS sample. Principal reagents were the U.S. Food and Drug Administration (FDA)-cleared Variant II Hemoglobin A1C Program (FDA K070452 and FDA K130860) obtained from Bio-Rad. DBS assay results were verified by

comparing %HbA1c values obtained from 177 DBS samples versus DBS-matched blood samples (all samples analyzed on the Variant II). The correlation coefficient of the linear regression (LR) comparison was $R^2 = 0.99$. Coefficient of Variation (CV) from Bio-Rad quality control (QC) samples and DBS QC samples assayed 75 over 20 months was:

Sample	Mean %HbA1c	Standard Deviation	Intra-Assay CV	Inter-Assay CV
Bio-Rad normal %HbA1c QC	5.3	0.1	1.1%	2.2%
Bio-Rad high %HbA1c QC	10.1	0.2	0.8%	2.3%
DBS normal %HbA1c QC	5.5	0.2	2.5%	2.8%

Per Bio-Rad directive, the reportable measurement range of the Variant II is 3.1% HbA1c to 20.0% HbA1c. Out of range high values are recoded to these values.

DBS Total Cholesterol (CHO) Assay. The DBS total cholesterol (**CHO**) microtiter plate assay was performed using conventional clinical chemistry reactions optimized to accommodate the limited microliter volume available from a DBS sample. Principal reagents were the FDA-cleared Synermed Cholesterol Reagent Kit (FDA K903015) obtained from Infrared Laboratory Systems (Westfield, IN) supplemented with Amplex Red (10-Acetyl-3,7-dihydroxyphenoxazine) obtained from Cayman Chemical (Ann Arbor, MI). DBS assay results were verified by comparing CHO concentrations obtained from 141 DBS samples analyzed by the DBS microtiter plate assay versus DBS-matched plasma samples analyzed by a UniCel DxC 800 Synchron Access Clinical System (Beckman Coulter, Brea, CA). The correlation coefficient of the linear regression comparison was $R^2 = 0.82$. CV of DBS CHO QC samples assayed 58 times over 10 months was:

Sample	Mean CHO, mg/dL	Standard Deviation	Intra-Assay CV	Inter-Assay CV
DBS low CHO QC	131	10	5.2%	7.9%
DBS high CHO QC	270	14	6.0%	5.2%

The assay lower limit of detection (**LLOD**) and upper limit of detection (**ULOD**) were DBS direct CHO 15mg/dL and 1024mg/dL, respectively. If the NHANES equivalent was lower than 59mg/dL it was recoded to this the lower reportable limit. Nine cases were recoded.

DBS High Density Lipoprotein (HDL) Assay. The DBS HDL cholesterol (HDL) microtiter plate assay was performed using conventional clinical chemistry reactions optimized to accommodate the limited microliter volume available from a DBS sample. Principal reagents were the FDA-cleared Synchron Systems HDL Cholesterol Model A15625 System (FDA K042195) obtained from Beckman Coulter supplemented with Amplex Red (10-Acetyl-3,7-dihydroxyphenoxazine) obtained from Cayman Chemical. DBS assay results were verified by comparing HDL concentrations obtained from 66 DBS samples analyzed by the DBS microtiter plate assay versus DBS-matched plasma samples analyzed by the UniCel DxC 800 Synchron Access Clinical System. The correlation coefficient of the LR comparison was $R^2 = 0.91$. CV of DBS QC samples assayed 58 times over 10 months was:

Sample	Mean HDL, mg/dL	Standard Deviation	Intra-Assay CV	Inter-Assay CV
DBS low HDL QC	4	4	5.0%	9.7%
DBS high HDL QC	98	8	3.8%	7.7%

The assay LLOD and ULOD were DBS direct HDL 7mg/dL and 128mg/dL, respectively. The NHANES equivalent value of HDL 11mg/dL is considered to be the lower reportable limit. Samples with lower NHANES equivalent values (17 cases) are recoded to this value; there are no high OOR cases.

DBS C-Reactive Protein (CRP) Assay. The DBS C-reactive Protein (**CRP**) assay was performed by enzyme-linked immunosorbent assay (**ELISA**) optimized to accommodate the limited microliter volume available from a DBS sample. Principal reagents were the FDA-cleared BioCheck High Sensitivity C-Reactive Protein Enzyme Immunoassay Test Kit (FDA K003851) obtained from Percipio Biosciences (Manhattan Beach, CA). DBS assay results were verified by comparing CRP concentrations obtained from 146 DBS samples analyzed by the DBS microtiter plate ELISA versus DBS-matched plasma samples analyzed by an AU680 Clinical Chemistry Analyzer (Beckman Coulter). The correlation coefficient of the LR comparison was $R^2 = 0.89$ for DBS with direct CRP ≤5mg/L. Any sample with a DBS direct CRP >5mg/L was diluted and reassayed. The correlation coefficient of the LR comparison

was R^2 = 0.90 for DBS with direct CRP >5mg/L CV of DBS CRP QC samples assayed 76 times over 10 months was:

Sample	Mean CRP, mg/L	Standard Deviation	Intra-Assay CV	Inter-Assay CV
DBS low CRP QC	0.57	0.04	5.8%	7.7%
DBS high CRP QC	2.56	0.25	6.9%	9.7%

The assay LLOD and ULOD were DBS direct CRP 0.02mg/L and 5mg/L, respectively. The LLOD was beneath CRP 0.13mg/L considered to be the lower reportable limit. One sample with an NHANES equivalent value lower than this is recoded to 0.13.

DBS Cystatin C (CYS) Assay. The DBS cystatin C assay was performed by ELISA optimized to accommodate the limited microliter volume available from a DBS sample. Principal reagents were the Human Cystatin C ELISA Kit obtained from BioVendor (Asheville, NC). Performance of the ELISA has been standardized to the European Certified Reference Material for Cystatin C in Human Serum ERM-DA471/IFCC. DBS assay results were verified by comparing CYS concentrations obtained from 139 DBS samples analyzed by the DBS microtiter plate ELISA versus DBS-matched plasma samples analyzed by a conventional microtiter plate ELISA. The correlation coefficient of the LR comparison was R^2 = 0.79. CV of DBS CYS QC samples assayed 106 times over 14 months was:

Sample	Mean CYS, mg/L	Standard Deviation	Intra-Assay CV	Inter-Assay CV
DBS low CYS QC	1.01	0.07	3.4%	7.3%
DBS high CYS QC	1.77	0.11	3.9%	6.1%

The LLOD and ULOD of the DBS microtiter plate assay were DBS direct CYS 0.14mg/L and 7mg/L, respectively; 0.37mg/L considered to be the lower reportable limit. 55 samples with a DBS direct CYS concentration equating to a NHANES equivalent concentration beneath the lower reportable limit is recoded to 0.37mg/L.

Description of 2016 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 where we can. We normally use data from the National Health and Nutrition Examination Survey (NHANES) to do this. We have constructed and release a variable for each assay, which we call an NHANES equivalent value. **While the original assay values are provided, we recommend the 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 a report, "Results from the Health and Retirement Study Biomarker Validation Project." 2013. Crimmins, E., Kim, J.K., McCreath, H., Seeman, T.; Validation of Blood-based Assays using Dried Blood Spots for use in Large Population Studies. 2014. Crimmins, E., Kim, J.K., McCreath, H., Faul, J., Weir, D., Seeman, T. Biodemography and Social Biology, 60: 38-48; and the HRS Documentation for the 2006 and 2008 blood-based assays. 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 2016 HRS data are compared to NHANES data from 2013-2016 for total cholesterol, HDL and hba1c; 2015-2016 for CRP; and 1999-2002 for Cystatin C. 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. NHANES data on CRP became available again in 2015-2016. So HRS 2014 equivalent values are based on NHANES 2009-2010 and HRS 2016 values are based on NHANES 2015-2016. Because cystatin C has not been regularly done in NHANES, HRS 2006, 2008, 2010, 2012, 2014 and 2016 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 to 2016 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 (PBIOWGTR). 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 2013-14 and 2015-16 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 2016 CRP we use the 2015-16 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.

NHANES Equivalent Values for HRS 2016 DBS Biomarkers

We have created NHANES equivalent variables of total cholesterol, HDL, HbA1c, CRP and Cystatin C using NHANES data (selected for 50+ and no weight missing) -NHANES 2013/14-2015/16 for HDL, TC, A1c; NHANES 2015/16 for CRP (2013/14 CRP is not available); NHANES 1999-2002 for Cystatin C.

We added small random numbers to the original values, renamed variables, then created percentiles. We run regressions to create equations; and use the constant and coefficients to adjust HRS 2016 values to NHANES equiv values.

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

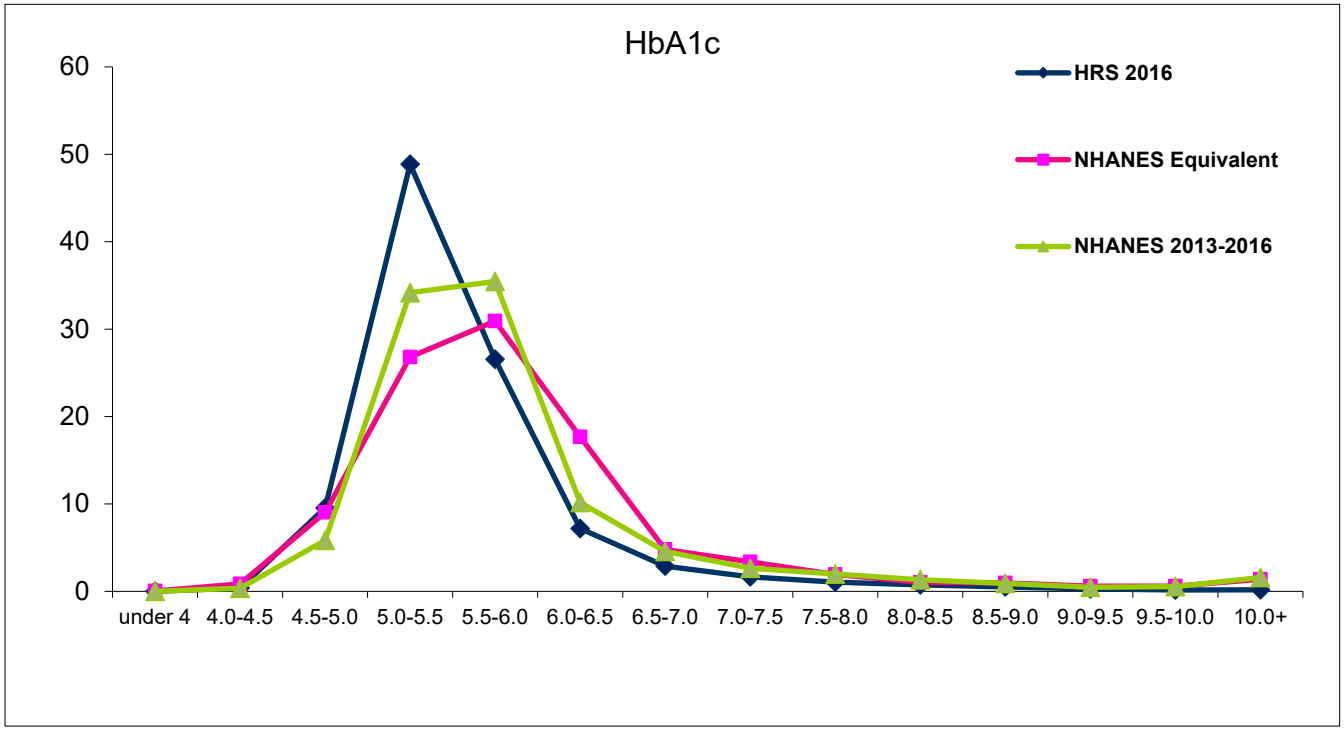
```
PA1C_ADJ = -2.63552 + HbA1c_dbs_num * 1.518781;
PTC_ADJ = 22.30231 + tc_dbs_num * 0.614378;
PHDL_ADJ = -2.524155 + HDL_dbs_num * 0.9410586;
PCYSC_ADJ = -0.2280132 + CYC_dbs_num * 1.044591;
PCRP_ADJ=exp(-0.0058354 + 0.9820371*log(crp_dbs_num));
```

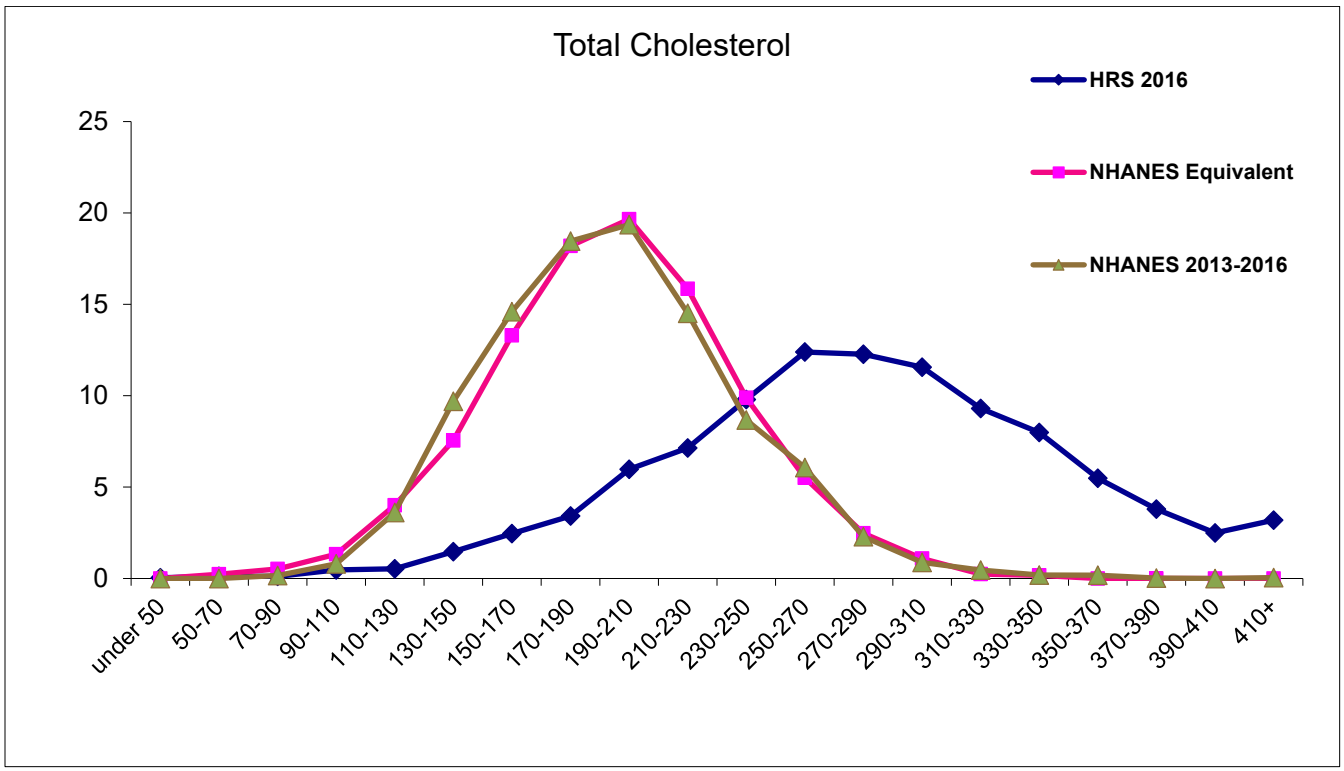
Because some values of cystatin C are less than 0, they are recoded to the lowest value in the data set.

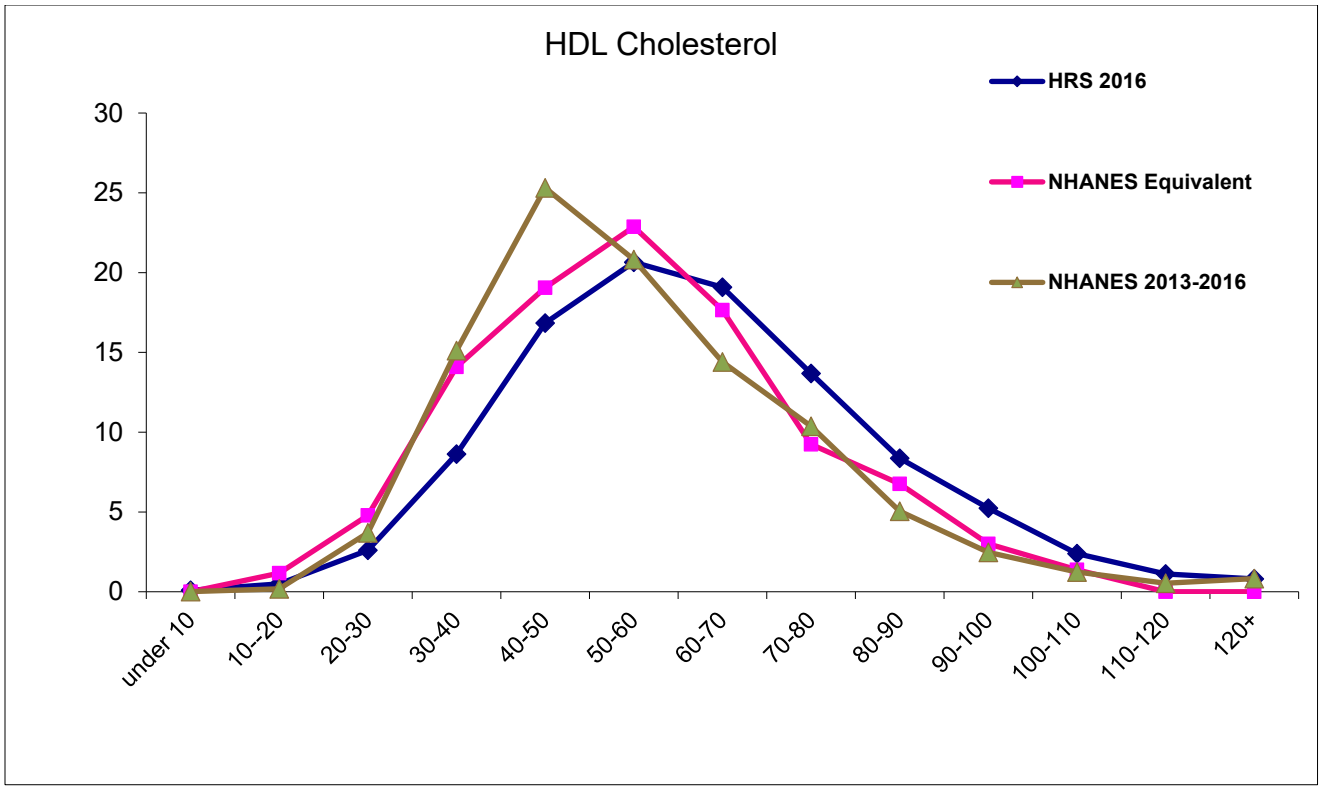
Description of HRS Blood-Based 2016 DBS Biomarker data

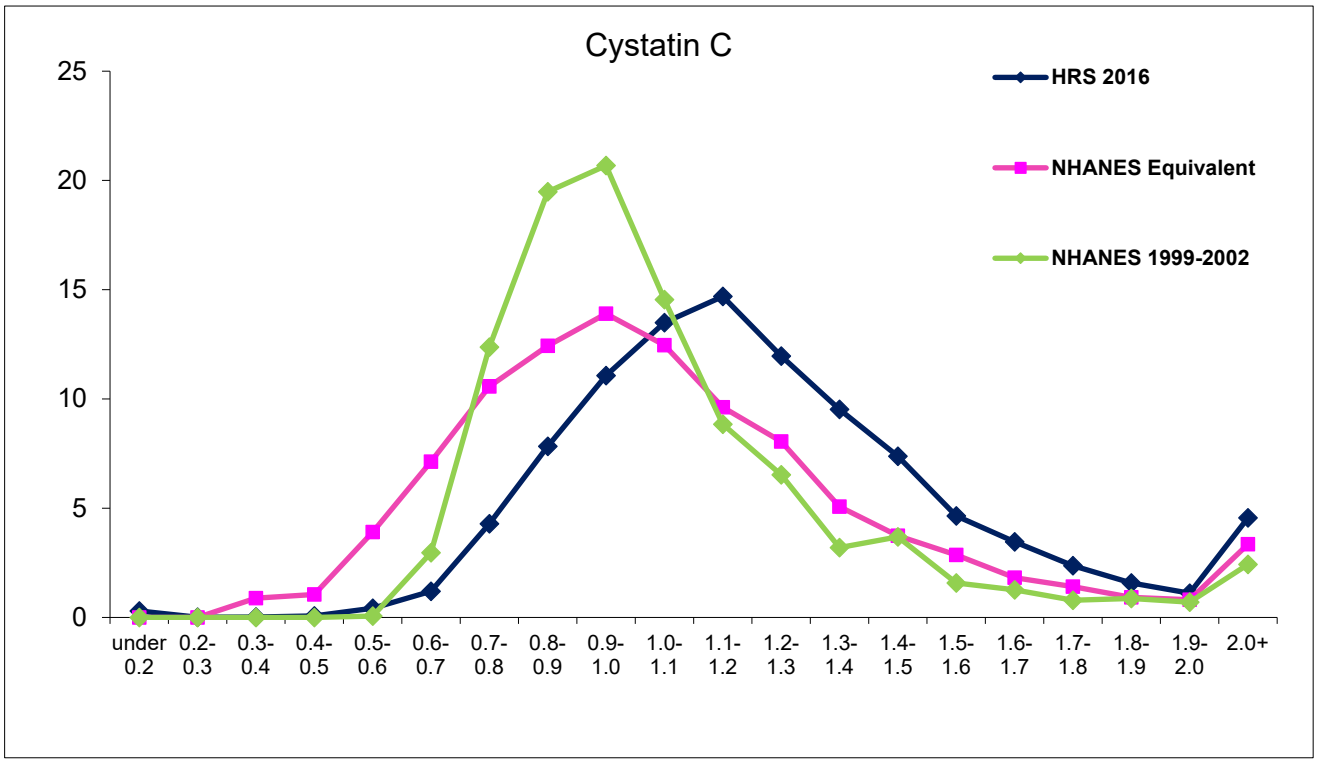
	N	Mean	SD	Min	Max
HRS Original value from DBS 2016 assay					
HbA1C (%) HbA1c_dbs_num	6177	5.62	0.70	3.90	17.10
Total Cholesterol (mg/dL) tc_dbs_num	6761	281.35	68.30	19.00	580.00
HDL (mg/dL) HDL_dbs_num	6636	62.67	20.01	7.00	128.00
Cystatin C (mg/L) cyc_dbs_num	6862	1.26	0.48	0.14	7.00
CRP (ug/mL) CRP_dbs_num	6869	3.79	4.71	0.09	20.00
Logged CRP Logcrp	6869	0.69	1.19	-2.41	3.00
NHANES (2013-2016)					
HbA1C (%)	5193	5.93	1.11	3.50	17.50
Total Cholesterol (mg/dL)	5131	195.08	43.28	82.00	540.00
HDL (mg/dL)	5131	56.45	19.78	6.00	226.00
Cystatin C(1999-2002) (mg/L)	3285	1.08	0.50	0.43	10.70
CRP (2015-2016) (ug/mL)	2543	3.56	4.24	0.08	20.00
Log CRP (2015-2016)	2543	0.66	1.19	-2.53	3.00
HRS 2016 NHANES Equivalent Value					
HbA1C (%) PA1C_ADJ	6177	5.91	1.07	3.29	20.00
Total Cholesterol (mg/DL) PTC_ADJ	6761	195.17	41.94	59.00	378.64
HDL (mg/dL) PHDL_ADJ	6636	56.46	18.81	11.00	117.93
Cystatin C (mg/L) PCYSC_ADJ	6862	1.08	0.49	0.37	7.08
CRP (ug/mL) PCRP_ADJ	6869	3.65	4.44	0.13	18.84
Logged CRP	6869	0.67	1.15	-2.04	2.94

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






HDL Cholesterol
HRS 2016
NHANES Equivalent
NHANES 2013-2016
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 2016
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
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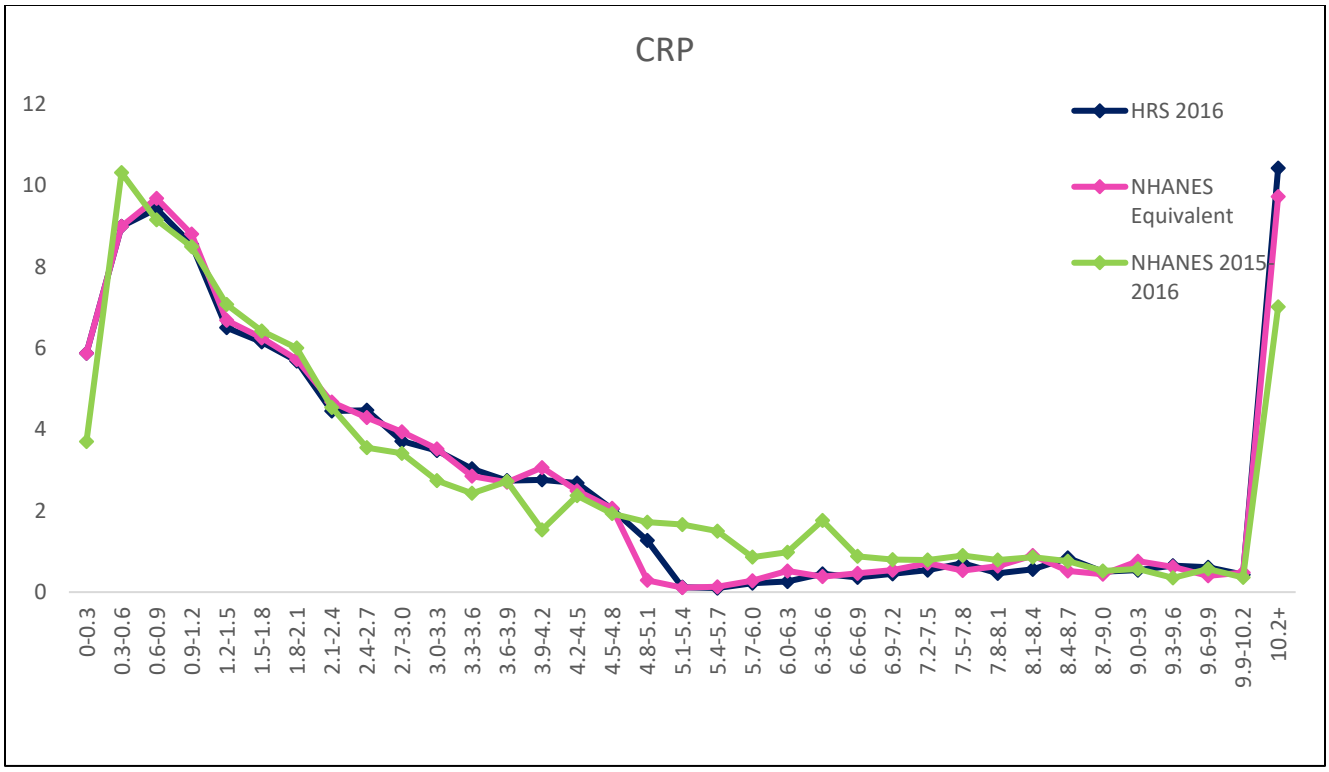
1.7- 1.8
1.8- 1.9
1.9- 2.0
2.0+


CRP
12
10
8
6
4
2
0
HRS 2016
NHANES Equivalent
NHANES 2015-2016
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
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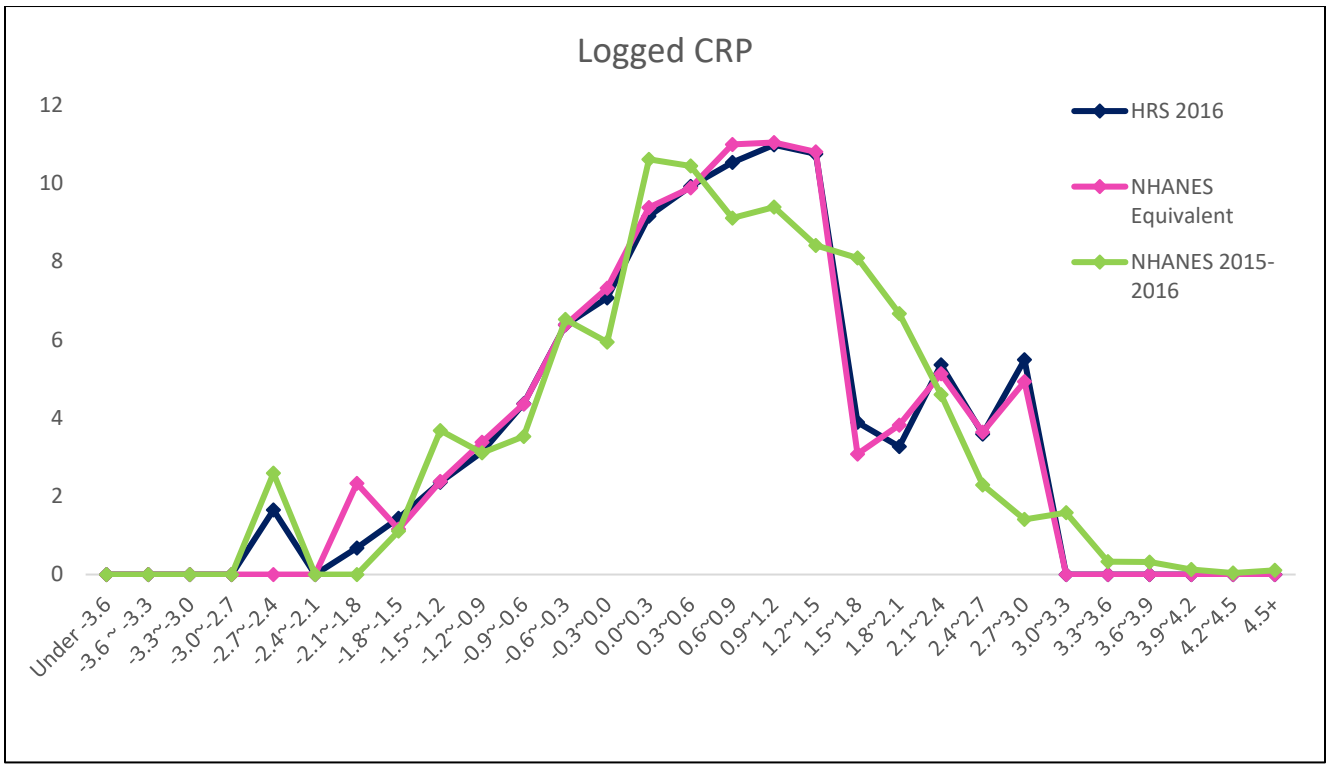
9.3-9.6
9.6-9.9
9.9-10.2
10.2+


Logged CRP
12
10
8
6
4
2
0
HRS 2016
NHANES Equivalent
NHANES 2015-2016
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+