

**HRS Documentation Report**

***Venous blood collection and assay protocol in the 2016 Health and Retirement Study***

***2016 Venous Blood Study (VBS)***

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## Overview

### Rationale

The HRS has emerged as the preeminent US study of its aging population because of its large sample size, significant oversamples of minorities, high response rates, quality of measurement, longitudinal design, and commitment to public use of data. Integration of biological measurement into HRS has been a signal innovation of the past 10+ years. Beginning in 2006, HRS collected physical performance measures, anthropometric measures, blood-based biomarkers from fingersticks and dried blood spots (DBS), and salivary DNA. These were collected on a rotating basis for half the sample at each wave through 2016 as part of a new enhanced face-to-face (EFTF) interview by trained survey interviewers. Each half-sample has now had up to three opportunities for this biomarker interview, alternating with three telephone interviews.

A competing revision award allowed HRS to collect venous blood for the first time in 2016 from panel respondents in a separate home visit by a trained phlebotomist in order to provide a substantially fuller picture of the health of a representative sample of older people. This has largely been accomplished and much of the assay work is done at this point. Most of the core assays have been completed on the full sample and are described in this document. We are planning to release and provide further documentation on additional assays on the whole sample in the spring of 2018. The assays for the Innovative subsample (described below) will also be released with documentation in 2018.

Some very innovative measurement of health status is resulting from this undertaking. HRS will have a unique ability to characterize immune function in the older population with the results of flow cytometry. We will also produce a unique data set for a large representative sample with indicators of DNA methylation, RNAseq, and DNA mitochondrial copy number. We will have assays that indicate functioning of multiple systems as well as multiple indicators of physiological dysregulation.

### Sample

All panel respondents who completed an HRS interview during the 2016 wave were asked to consent to a venous blood draw with the exception of proxy respondents and nursing home residents. The request was made by their HRS interviewer at the end of the interview (telephone and in person modes), with the offer of a fifty-dollar incentive payment to be sent by check. That incentive amount and procedure is consistent with other voluntary ancillary activities in HRS where we routinely pay in advance of completion. No higher amounts were offered.

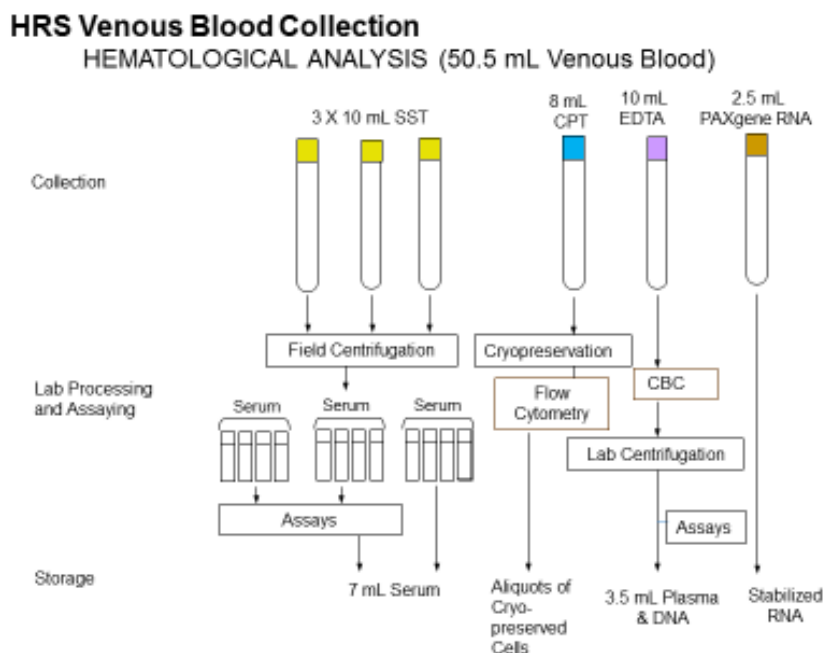
### Collection

The blood collection was managed by Hooper Holmes Health & Wellness. Hooper Holmes is a premiere biological-sample collection organization, collecting health information on more than 1.6 million people annually through a national network of thousands of medical professionals. The phlebotomy service was provided with the names, addresses, and phone numbers of consenting respondents and contacted respondents to set appointments. Collection materials were mailed to the phlebotomists' homes in advance of the scheduled visit. The phlebotomy service reported to HRS on respondents who declined to schedule appointments or missed scheduled appointments, and HRS staff followed-up to detect any problems and to attempt to reschedule appointments. Every attempt was made to schedule the blood draw within 4

weeks of the HRS core interview. Fasting was recommended and preferred but not required. Phlebotomists noted the fasting status of the samples.

We collected 50.5 mL of blood in 6 tubes – 1 8 mL CPT tube, 3 10 mL double gel serum separator tubes (SST), 1 10 mL EDTA whole blood tube, and a 2.5 mL PAXgene RNA tube. The SST tubes are centrifuged in the field before being shipped overnight to the CLIA-certified Advanced Research and Diagnostic Laboratory at the University of Minnesota. Tube processing is done within 24 hours of arrival at the lab (within 48 hours of collection).

A description of our blood collection is captured in the following figure.



## Participation

Participation by respondents to the blood collection protocol in 2016 was excellent, despite the need for a two-stage consent process which experience has shown to be a negative for response rates. Our final VBS sample with collected data was n=9934. The consent rate was 78.5% for cases interviewed through September 15, 2017 and of these, 82.9% had a completed collection for a final completion rate of 65% among eligible cases. Final completion rates among eligible cases are shown in the table below.

Age	Completion %	Race/Eth	Completion %	Education	Completion %
< 65	64.2%	White	67.9%	< 12 Years	65.0%
65-79	66.3%	Black	57.3%	High School / Some College	64.7%
80+	64.2%	Hispanic	65.2%	College +	65.8%

## Assays

As a multidisciplinary population survey serving a wide community of researchers, the decision process in HRS about any survey content, including biomarkers, must consider a wide range of potential uses and not focus narrowly on specific hypotheses or interests. Input was sought from a group of about 30 experts, including several meetings of the NIA Data Monitoring Committee, NIA scientific advisors, and researchers in aging and immunology and biology of aging. The choice of measures attempted to balance scientific value against cost and respondent burden. In addition, assay selection was driven by what is feasible given the limitations of the blood collection methods (volume, processing, and shipping) and collection on a dispersed national sample. Several assays were not included because laboratory guidelines require the specimen be centrifuged, separated and frozen immediately, require special handling (light protection), or have circadian variation that would interfere with interpretation of analyte measurements (e.g. thyroid stimulating hormone).

Biomarkers serve two main purposes in surveys. One is to improve the measurement of health status by providing observations on health conditions that are not easily observed and reported by respondents. The other is to help elucidate pathways connecting social and environmental factors to broad health outcomes. The markers selected as part of the 2016 Venous Blood Study address one or both of these goals. We proposed to assay some conventional blood-based biomarkers for harmonization with other studies and because of their relevance to aging-related clinical outcomes and we also proposed some measures that are novel for a population-based study of this size and representativeness including a proposed panel of cytokines, immune system markers, molecular and cellular markers of aging, and gene expression. We also devote a substantial part of the blood collection to storage in repository to take advantage of future scientific and technological developments.

A summary of assays by collection tube is presented in the table below.

Venous Blood Study Assays and Estimated Storage by Collection Tube		
Tube type and blood volume	Assay	Storage
7.5mL SST (Serum) - 1	Total cholesterol, HDL and LDL cholesterol, Triglycerides, High sensitivity CRP (hsCRP), Vitamin D (25 Hydroxy), Ferritin (FRTN), IGF-1, DHEAs, Cytokine Panel (IL-6, IL-1RA, IL-10, TNF-alpha, sTNFR-I, and TGF beta [activated form]), B-type natriuretic peptide (NT-proBNP)	8 mL Serum
7.5mL SST (Serum) - 2	Comprehensive Metabolic Panel ([CMP]: Albumin, Alk Phos, ALT, AST, Bili, Ca, Cl, CO2, Creat, Gluc, K, Total protein, Na, BUN), Homocysteine, Cystatin C, Clusterin, BDNF	
7.5mL SST (Serum) - 3	Repository only	
8 mL CPT	Cryopreserved cells / Flow cytometry	8 Aliquots of cryopreserved cells

10 mL EDTA (Whole Blood)	Complete blood count ([CBC]: White blood cell [WBC, leukocyte] count, White blood cell types [WBC differential], Red blood cell [RBC] count, Hematocrit [HCT, packed cell volume, PCV], Hemoglobin [Hgb], Red blood cell indices: mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], and mean corpuscular hemoglobin concentration [MCHC], Platelet [thrombocyte] count, Mean platelet volume [MPV]), CMV seroprevalence, mtDNA copy number, telomere length, DNA methylation, <b>HbA1c</b> **	3.5 mL plasma Buffy coat Extracted DNA
2.5 mL Paxgene RNA	RNA-seq	Stabilized RNA

\* Assays in bold are included in 2018-2023 protocol

\*\* HbA1c added for 2018-2023

### Assays on whole sample

Not all assays were done on the entire sample; some were done on a selected subsample (see below). Results of 27 assays (listed below) are provided to respondents within 2-4 weeks of collection. Assays were reported if the lab was CLIA certified for the assay and it is regarded as a clinically relevant assay. Indicators based on flow cytometry were done on the entire sample but these are listed in a separate section below.

### Alert values

Results of some assays are monitored in real time and respondents are alerted by a team physician within 24 hours of any severely out of expected range results. The following table lists the assays that are monitored for alert values.

Test	Alert Value
Potassium	<2.7 or >6.0 mmol/L
Sodium	<120 or >160 mmol/L
WBCs	<1.0 or >50 x 10 <sup>9</sup> /L
Serum Creatinine	>= 2.4 mg/dL

### Assays Measured on Whole Sample: Reported and Not Reported to Respondents

A. Reported to Respondents			
Lipid panel	Comprehensive Metabolic Panel		Complete Blood Count
Total cholesterol	Fasting glucose	Aspartate aminotransferase	Red blood cell count
LDL cholesterol	Creatinine	Bilirubin	Red cell distribution
HDL cholesterol	Blood urea nitrogen	Calcium	Platelets
Triglycerides	Albumin	Total protein	Corpuscular volume
	Alkaline phosphatase	Sodium	Hemoglobin concentration
	Alanine aminotransferase	Potassium	Hematocrit
		Chloride	Hemoglobin
			Ferritin
			White blood cell count

<b>B. Not Reported Back to Respondents</b>	
High sensitivity CRP (hsCRP)	Eosinophil count
Cystatin C	Basophil count
% Neutrophils	IGF-1
% Lymphocytes	DHEA-S
% Monocytes	Flow cytometry (cryopreserved cells)
% Eosinophils	CMV seroprevalence
% Basophils	B-type natriuretic peptide NT-proBNP
Neutrophil count	Vitamin D
Lymphocyte count	Cytokines: IL-6, IL-1RA, IL-10, TNF-alpha, sTNFR-I, TGF beta (activated form)
Monocyte count	

### Subsample assays

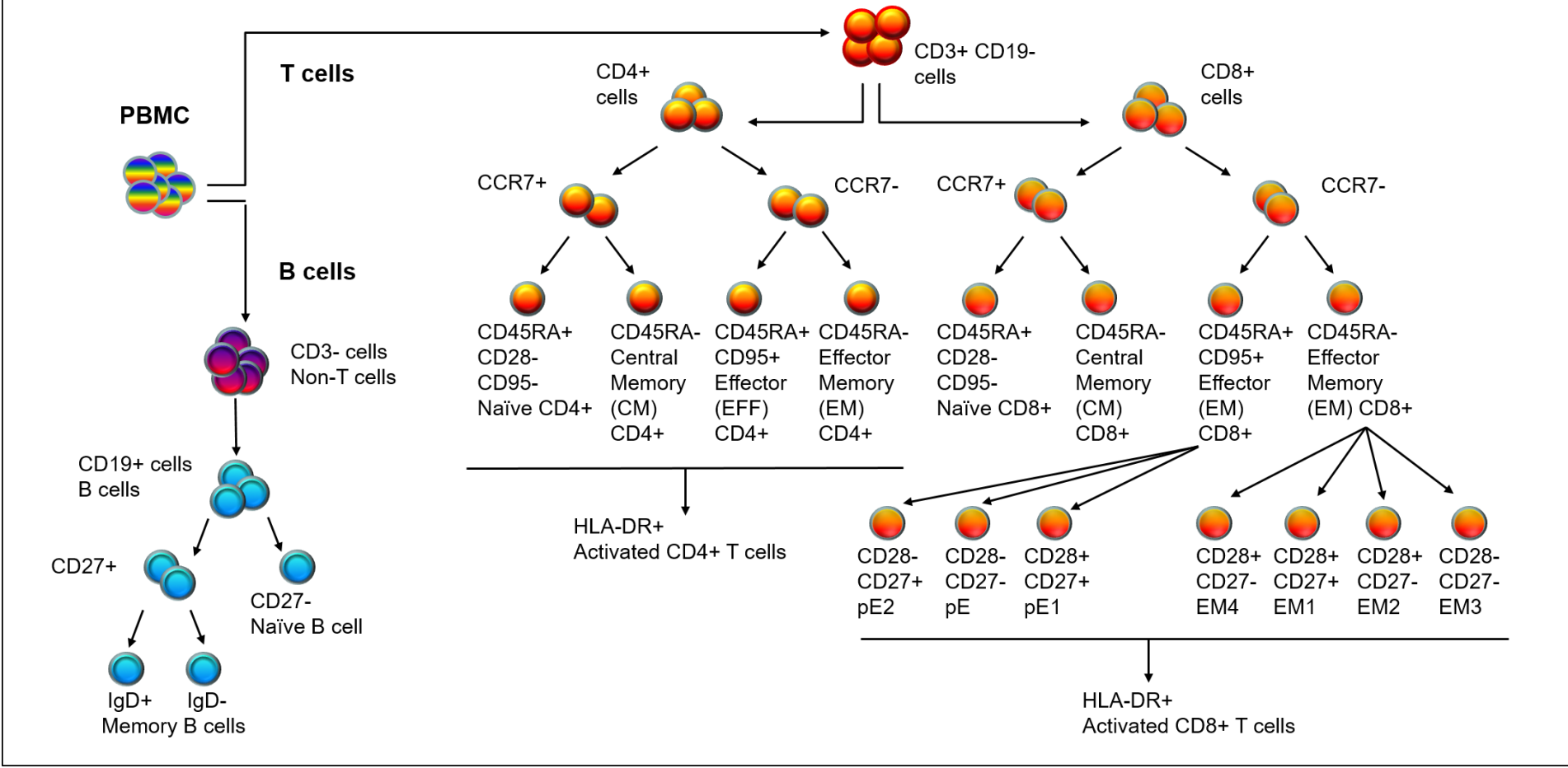
We have identified a random sample of approximately 4,000 HRS respondents with venous blood samples for whom we are now performing a set of innovative assays reflecting basic molecular, cellular, and epigenetic processes of aging including mitochondrial DNA copy number, telomere length, RNA-seq, and DNA methylation. The sample includes all the participants of the 2016 Healthy Cognitive Aging Project (HCAP) who have provided blood samples, plus younger participants designated for future HCAP assessments, and a subsample of HCAP non-participants. This subsample fully represents the entire HRS sample. Information on many of these innovative markers is not available for a nationally representative sample of Americans, nor for a significant number of race/ethnic minority respondents in any sample. Data from this innovative sample will allow us to assess the value of these assays in adding to our explanatory power for health outcomes related to age and social and environmental exposures, and guide future planning for assay work as we build the longitudinal repository. These assay results are expected to be released in 2018 with further documentation. Assays performed on the subsample only include:

<b>Assays on Subsample only</b>
Telomere length
mtDNA
Methylation
Homocysteine
RNAseq
Clusterin / ApoJ
Brain-derived neurotrophic factor (BDNF)

### Flow cytometry measures

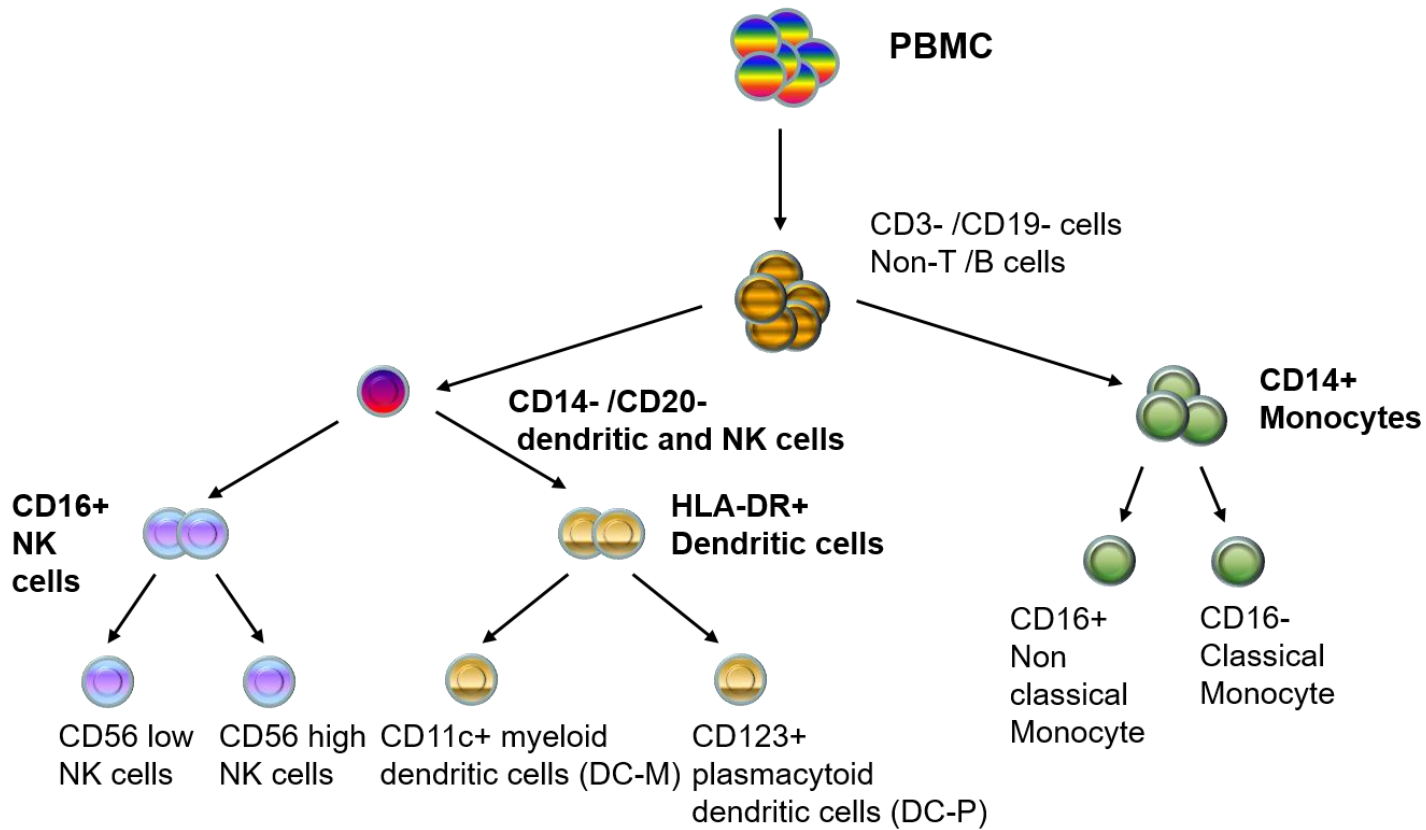
Our approach to including flow cytometry was developed after multiple pilot studies to clarify feasibility and the effects of our collection and shipping procedures. In addition, the assay procedure was developed with substantial input from Janko Nikolich-Zugich. We use flow cytometry to characterize the distributions of 33 different types of T, B, monocytes, and NK cells. Figures 1 and 2 below indicate the measured cell subsets – with counts and %. The table below indicates that variables that we will provide to users. In the results section we provide some simple descriptive results from selected measures for the first ~1,000 sample. The results for the entire VBS sample will be released in the future.

# B and T cells





# Monocytes, dendritic and natural killer cells



## Markers Determined through Flow Cytometry

CELL TYPE	MARKERS	PARENT POPULATION
B lymphocytes	CD3- CD19+	single, live lymphocytes
IgD+ memory B cells	CD3- CD19+ IgD+ CD27+	Bcell
IgD- memory B cells	CD3- CD19+ IgD- CD27+	Bcell
Naive B cells	CD3- CD19+ IgD+ CD27-	Bcell
T cells	CD3+ CD19-	single, live lymphocytes
Cytotoxic T cells	CD3+ CD19- CD8+ CD4-	T cells
Activated Cytotoxic T cells	CD3+ CD19- CD8+ CD4- HLA-DR+	Cytotoxic T cells
Central Memory cytotoxic T cells	CD3+ CD19- CD8+ CD4- CD45RA- CCR7+ CD28+ CD95+	Cytotoxic T cells
Effector (E) cytotoxic T cells	CD3+ CD19- CD8+ CD4- CD45RA+ CCR7-	Cytotoxic T cells
pE cytotoxic T cells	CD3+ CD19- CD8+ CD4- CD45RA+ CCR7- CD27- CD28-	Effector Cytotoxic T cells
pE1 cytotoxic T cells	CD3+ CD19- CD8+ CD4- CD45RA+ CCR7- CD27+ CD28+	Effector Cytotoxic T cells
pE2 cytotoxic T cells	CD3+ CD19- CD8+ CD4- CD45RA+ CCR7- CD27+ CD28-	Effector Cytotoxic T cells
Effector Memory (EM) Cytotoxic T cells	CD3+ CD19- CD8+ CD4- CD45RA- CCR7-	Cytotoxic T lymphocytes
EM1 cytotoxic T cells	CD3+ CD19- CD8+ CD4- CD45RA- CCR7- CD27+ CD28+	Effector Memory Cytotoxic T cells
EM2 cytotoxic T cells	CD3+ CD19- CD8+ CD4- CD45RA- CCR7- CD27+ CD28-	Effector Memory Cytotoxic T cells
EM3 cytotoxic T cells	CD3+ CD19- CD8+ CD4- CD45RA- CCR7- CD27- CD28-	Effector Memory Cytotoxic T cells
EM4 cytotoxic T cells	CD3+ CD19- CD8+ CD4- CD45RA- CCR7- CD27- CD28+	Effector Memory Cytotoxic T cells
Naïve cytotoxic T cells	CD3+ CD19- CD8+ CD4- CD45RA+ CCR7+ CD95- CD28+	Cytotoxic T cells
Helper T cells	CD3+ CD19- CD8-	T cells
Activated helper T	CD3+ CD19- CD8- CD4+ HLA-DR+	Helper T cells

cells		
Central Memory Helper T cells	CD3+ CD19- CD8- CD4+ CD45RA CCR7 CD28+ CD95+	Helper T cells
Effector Helper T cells	CD3+ CD19- CD8- CD4+ CD45RA CCR7 CD28 CD95	Helper T cells
Effector memory helper T cells	CD3+ CD19- CD8- CD4+ CD45RA- CCR7-	Helper T cells
Naïve Helper T cells	CD3+ CD19- CD8+ CD4- CD45RA+ CCR7+ CD95- CD28+	Helper T cells
Dendritic cells, Natural Killer lymphocytes, monocytes	CD3- CD19-	single, live PBMC
Dendritic cells, Natural Killer lymphocytes	CD3- CD19- CD20- CD14-	DC NK MONO
Dendritic cells	CD3- CD19- CD20- CD14- HLA-DR+	DC NK
Myeloid Dendritic cells	CD3- CD19- CD20- CD14- HLA-DR+ CD11c+ CD123-	DC
Plasmacytoid Dendritic cells	CD3- CD19- CD20- CD14- HLA-DR+ CD11c- CD123+	DC
Natural Killer lymphocytes	CD3- CD19- CD20- CD14- CD16+ CD56+	DC NK
CD56HI Natural Killer lymphocytes	CD3- CD19- CD20- CD14- CD16+ CD56HI	NK
CD56LO Natural Killer lymphocytes	CD3- CD19- CD20- CD14- CD16+ CD56LO	NK
MONOCYTES	CD3- CD19- CD20- CD14+	DC NK MONO
Classical monocytes	CD3- CD19- CD20- CD14+ CD16-	MONO
Non classical monocytes	CD3- CD19- CD20- CD14+ CD16+	MONO

## Laboratory

All assays are being done at the University of MN Advanced Research and Diagnostic Laboratory (ARDL) under the direction of Bharat Thyagarajan. The ADRL is CLIA certified and was last inspected in August 2015. Facilities include a 17,000 square foot specialized laboratory and 2,000 square feet of office space. Advanced Research and Diagnostic Laboratory (ARDL) was established about 30 years ago for improved coordination and centralization of laboratory activities of multi-center research studies contracted by the Department of Laboratory Medicine and Pathology at the University of Minnesota.

### Laboratory Equipment

*Roche Cobas 6000:* This instrument is a high throughput random access analyzer for routine chemistry and immunology assays.

*Roche Cobas e411 Analyzer* This instrument is a high throughput analyzer used for Immunoassays.

*Tosoh G8 Automated HPLC Analyzer:* This is a fully automated, compact benchtop analyzer used for rapid HbA1c measurement.

*Siemens BN Prospec and BNII:* This instrument is used for plasma protein nephelometry.

*SciEX 6500 Qtrap LC MS/MS:* This instrument is a liquid chromatography/ mass spectrometry system.

*Sysmex XS1000i:* This instrument is an automated hematology analyzer utilizing fluorescent flow cytometry.

*Viacell:* This instrument is an automated cell counter purchased for the HRS study.

*Protein Simple ELLA System:* This is a microfluidic based ELISA platform purchased for the HRS study.

*Beckman Coulter Biomek NXP Automated Laboratory Workstation:* This instrument is used to assist in performance of ELISA assays.

*Molecular Devices SpectraMax Spectrophotometer:* This is a plate reader spectrophotometer for reading ELISA plates manually.

*Hamilton Easy Blood System:* This instrument is an automated liquid handler for sample processing that was purchased for the HRS study.

*AutoGen FlexSTAR:* This instrument provides automated DNA extractions.

*QiaSymphony:* This instrument provides automated RNA extractions and was purchased for the HRS study.

*Agilent TapeStation:* This instrument provides quality analysis and quantitation of RNA extractions and was purchased for the HRS study.

*Biorad CFX Real Time System:* This is a real-time PCR instrument purchased for the HRS study.

*Flow Cytometry instrument:* BD biosciences LSRII; purchased a BD Biosciences LSR Fortessa for the HRS study.

## Description of Assays Performed in the Entire VBS Cohort

**Albumin:** Albumin is measured in serum on a Roche Cobas 6000 Chemistry Analyzer (Roche Diagnostics Corporation) using a bromcresol purple method (Roche Diagnostics, Indianapolis, IN). The laboratory inter-assay CV is 2.2% at a concentration of 2.67 g/dL and 2.6% at a level of 3.90 g/dL.

**Alkaline Phosphatase:** Alkaline Phosphatase is measured in serum on a Roche Cobas 6000 Chemistry Analyzer (Roche Diagnostics Corporation) using a colorimetric method (Roche Diagnostics, Indianapolis, IN). This method was endorsed by the International Federation of Clinical Chemistry in 1983. The laboratory inter-assay CV is 1.7% at a concentration of 264 U/L and 3.3% at a concentration of 215 U/L.

**Alanine Aminotransferase (ALT):** Alanine aminotransferase is measured in serum on a Roche COBAS 6000 Chemistry Analyzer (Roche Diagnostics Corporation) using an alpha-ketoglutaratic enzymatic method (Roche Diagnostics, Indianapolis, IN). The laboratory inter-assay CV is 2.4% at a concentration of 21 U/L and 2.0% at a concentration of 133 U/L.

**Aspartate Aminotransferase (AST):** Aspartate aminotransferase is measured in serum on a Roche COBAS 6000 Chemistry Analyzer (Roche Diagnostics Corporation) using an alpha-ketoglutaratic enzymatic method (Roche Diagnostics, Indianapolis, IN). The laboratory inter-assay CV is 2.4% at a concentration of 21 U/L and 3.3% at a concentration of 141 U/L.

**B-type natriuretic peptide, N-terminal pro (NT-proBNP):** NT-proBNP is measured in serum on a Roche e411 Analyzer (Roche Diagnostics Corporation) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN). The laboratory inter-assay CV is 3.3% at a concentration of 140 pg/mL and 2.8% at a concentration of 4707 pg/mL.

**Bilirubin (Total and Direct):** Total (direct+ indirect) bilirubin is measured in serum using a diazonium salt/ion colorimetric assay (Roche Diagnostics, Indianapolis, IN 46250) and read on the Roche Cobas 6000 Chemistry Analyzer (Roche Diagnostics). The reference range is 0.2 – 1.3 mg/dL with an inter-assay CV of 3.1%. Direct bilirubin is measured in serum on the Roche Modular P Chemistry Analyzer using a colorimetric assay based on the diazo Jendrassik-Grof procedure (Roche Diagnostics). The reference range is 0 – 0.2 mg/dL. The laboratory inter-assay CV is 7.3% at a concentration of 0.26 mg/dL and 3.3% at a concentration of 3.66 mg/dL.

**Bicarbonate (CO<sub>2</sub>):** Bicarbonate is measured in serum using Roche CO<sub>2</sub>-L reagent (Roche Diagnostics, Indianapolis, IN) on the COBAS 6000 Chemistry analyzer (Roche Diagnostics Corporation). The laboratory inter-assay CV is 4.4% at a concentration of 23.3 mmol/L and 7.4% at a concentration of 29.5 mmol/L.

**C-reactive Protein, High Sensitive (hsCRP):** hsCRP is measured in serum using a latex-particle enhanced immunoturbidimetric assay kit (Roche Diagnostics, Indianapolis, IN 46250) and read on the Roche COBAS 6000 Chemistry analyzer (Roche Diagnostics). The reference range is 0 – 5 mg/L. The laboratory inter-assay CV is 5.1% at a concentration of 1.05 mg/L and 6.7% at a concentration of 3.12 mg/L.

**Calcium:** Calcium is measured in serum a Roche COBAS 6000 Chemistry Analyzer (Roche Diagnostics Corporation) using a colorimetric method. The laboratory inter-assay CV is 1.3% at a mean concentration of 5.84 mg/dL and 1.3% at a concentration of 14.15 mg/dL.

**CBC (Complete Blood Count):** Hemogram, differential and platelet count are measured in EDTA whole blood using a Sysmex XE-2100 instrument, (Sysmex America, Inc., Lincolnshire, IL). Laboratory CV varies by assay.

**Cholesterol:** Total cholesterol is measured in serum using a cholesterol oxidase method (Roche Diagnostics, Indianapolis, IN 46250) on a Roche COBAS 6000 Chemistry Analyzer (Roche Diagnostics Corporation). The calibration of this assay is regularly monitored by the CDC/NHLBI Lipid Standardization Program. The NCEP program recommends reference range of <200 mg/dL. The laboratory inter-assay CV is 2.1% at a concentration of 180 mg/dL and 2.2% at a concentration of 260 mg/dL.

**HDL (High Density Lipoprotein)-Cholesterol:** HDL-cholesterol is measured directly in serum using the Roche HDL-Cholesterol 3<sup>rd</sup> generation direct method (Roche Diagnostics, Indianapolis, IN) on a Roche Cobas 6000 Chemistry Analyzer (Roche Diagnostics Corporation). This method is standardized against the designated CDC reference method; and calibration of the assay is regularly monitored by the CDC/NHLBI Lipid Standardization Program. The NCEP program recommends a reference range of >40 mg/dL. The laboratory inter-assay CV is 1.3% at a mean concentration of 26.6 mg/dL and 1.3% at a mean concentration of 51.3 mg/dL.

**LDL (Low Density Lipoprotein)-Cholesterol:** LDL-cholesterol is calculated in serum specimens having a triglyceride value <400 mg/dL using the formula of Friedewald LDL = TC - HDL - TG/5.0 (mg/dL), (Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499-502). The NCEP program recommends reference range of <100 mg/dL.

**Triglyceride:** Triglyceride is measured in serum using an enzymatic Triglyceride Reagent (Roche Diagnostics, Indianapolis, IN) on a Roche Cobas 6000 Chemistry Analyzer. (Roche Diagnostics Corporation). This assay performs no glycerol blank, and values agree with CDC proficiency testing since the majority of the laboratories do not use a glycerol blank. This method uses a manufacturer calibrator traceable to ID/MS method (NIST reference material SRM 1951. The NCEP program recommends reference range of <150 mg/dL. The laboratory inter-assay CV is 2.1% at a mean concentration of 112.4 mg/dL and 4.4% at a mean concentration of 195.8 mg/dL.

**CMV (IgG):** Method: CMV seroprevalence was measured using IgG antibodies to cytomegalovirus (CMV) in serum using the Roche e411 immunoassay analyzer (Roche Diagnostics Corporation, Indianapolis, IN). The Inter-assay CVs were 3.4% at a mean concentration of 1.23 COI (cutoff interval) and 2.9% at a mean concentration of 141.4 COI. The lower limit of detection is 0.15 COI. This is a qualitative assay. Results are reported as non-reactive (<0.5 COI), borderline (0.5 to <1.0 COI), or reactive ( $\geq$ 1.0 COI).

**Creatinine:** Creatinine is measured in serum by the Roche enzymatic method (Roche Diagnostics, Indianapolis, IN 46250) on a Roche COBAS 6000 Chemistry Analyzer. (Roche Diagnostics Corporation). The method is calibrated, and checked semi annually, using a National Institute of Standards and Technology (NIST) standard traceable to

reference material SRM 909b (Isotope Dilution Mass Spectroscopy (IDMS)). The laboratory CV is 2.9% at a concentration of 0.835 mg/dL and 2.8% at a concentration of 3.93 mg/dL.

**Cystatin C:** Cystatin C is measured in serum or plasma using Cystatin C reagent (Gentian, Moss Norway) on the Roche COBAS 6000 Chemistry analyzer. The laboratory inter-assay CV is 4.3% at a concentration of 0.75 mg/L and 3.2% at a concentration of 3.83 mg/L.

**Dehydroepiandrosterone Sulfate (DHEAs):** DHEAs is measured in serum using the Roche e411 Analyzer (Roche Diagnostics Corporation) using a competition immunoassay method (Roche Diagnostics, Indianapolis, IN). The laboratory inter-assay CV is 5.3% at 4.87  $\mu\text{mol/L}$  and 5.1% at 13.21  $\mu\text{mol/L}$ .

**DNA Isolation:** DNA is isolated from packed cells that are frozen at  $-70^{\circ}\text{C}$ . The DNA extraction and purification method uses a modified salt precipitation method for protein removal using commercial Flexigene® reagents (Qiagen Instrument Service, Germantown, MD) and a FlexStar+ Instrument (Autogen, Inc., Holliston, MA). DNA is quantitated using the NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Quantitation by Picogreen analysis (Molecular Probes, Eugene, OR) is also available. A mean yield of 200  $\mu\text{g}$  or 40  $\mu\text{g}$  DNA/mL packed cell is obtained, and DNA is of high quality (mean purity  $A_{260}/A_{280}=1.77$ ) and high molecular weight as determined by gel electrophoresis.

**Electrolytes:** Sodium, potassium, and chloride electrolytes are measured in serum by ion-selective electrode (ISE) method on the Roche Cobas 6000 Chemistry analyzer (Roche Diagnostics Corporation). The laboratory inter-assay CVs for sodium, potassium, and chloride are 1.0%.

**Ferritin:** Ferritin is measured in serum by a turbidimetric antibody method using Roche reagents (Roche Diagnostics Corporation) on the Roche COBAS 6000 instrument (Roche Diagnostics, Indianapolis IN). The laboratory CV for this assay is 9.9% at 42.2 ng/mL and 13.8% at 51.2 ng/ml.

**Glucose:** Glucose is measured in serum by the Roche hexokinase method (Roche Diagnostics, Indianapolis, IN) on a Roche Cobas 6000 Chemistry Analyzer (Roche Diagnostics Corporation). The method is calibrated and checked quarterly against Standard Reference Material 965 from National Institute of Standards and Technology (NIST) traceable to the NIST definitive method for glucose (Isotope Dilution Mass Spectroscopy (IDMS)). The laboratory inter-assay CV is 1.3% at a mean concentration of 97.2 mg/dL and 1.8% at a mean concentration of 223.3 mg/dL.

**Flow Cytometry Assays:** The immune cell subsets were identified using minor modifications to the standardized protocol published by the Human Immunology Project (Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. *Nat Rev Immunol.* 2012 Feb 17;12(3):191-200). The modifications include addition of CD95 to more definitively identify different subsets of effector cytotoxic T cells. One vial of cryopreserved mononuclear cells containing ~4 million cells was thawed and cells were incubated at  $37^{\circ}\text{C}$  in RPMI media for 1 hour. The cells were centrifuged at 1200rpm for 10 min at room temperature. The cells were resuspended in 1X PBS and stained using the two antibody cocktails as outlined in the tables below. The cells were kept on ice until analysis. All flow cytometry measurements

were performed on a LSRII flow cytometer or a Fortessa X20 instrument (BD Biosciences, San Diego, CA).

<b>Panel 1 (T cells and B cells)</b>				
Marker	Clone	Fluorochrome	Provider (cat #)	Volume/sample (µl)
brilliant stain buffer	NA	NA	BD (659611)	50
viability dye	NA	FVS 570 (PE)	BD (564995)	12
CD3	UCHT1	APC	BD (555335)	4
HLA-DR	G46-6	PE-CF594	BD (562331)	1
CD19	SJ25C1	PE-Cy7	BD (557835)	1
CD27	O323	FITC	biolegend (302806)	2.5
CD8	RPA-T8	BUV395	BD (563796)	1
IgD	IA6-2	BUV737	BD (564687)	1
CCR7	G043H7	BV421	biolegend (353208)	2.5
CD28	CD28.2	BV510	biolegend (302936)	2.5
CD95	DX2	BV605	biolegend (305628)	2.5
CD45RA	HI100	BV711	biolegend (304138)	2.5
CD4	RPA-T4	APC-Cy7	BD (557871)	1

<b>Panel 2 (monocytes, DC, NK)</b>				
Marker	Clone	Fluorochrome	Provider (cat #)	Volume/ sample (ul)
brilliant stain buffer	NA	NA	BD (659611)	50
viability dye	NA	FVS 570 (PE)	BD (564995)	12
CD3	UCHT1	APC	BD (555335)	4
HLA-DR	G46-6	PE-CF594	BD (562331)	1
CD19	SJ25C1	PE-Cy7	BD (557835)	1
CD11c		BB515	BD (564490)	1
CD20	2H7	BUV395	BD (563781)	1
CD16	3G8	BUV737	BD (564433)	1
CD56	NCAM16.2	BV421	BD (562751)	1
CD14	MOP9	BV510	BD (563079)	1
CD123	9F5	BV711	BD (563161)	1



CD45	2D1	APC-Cy7	BD (560178)	0.5
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**Insulin-Like Growth Factor 1 (IGF-1):** IGF-1 is measured in serum by an enzyme-linked immunosorbent assay (ELISA) technique using the Human Insulin-Like Growth Factor 1 Simple Plex Assay on the ELLA System from Protein Simple (San Jose, CA). The manufacturer inter-assay CV is 8.1, 8.3 and 7.5% at mean levels of 382, 967 and 1941 pg/uL. This assay has been correlated to the Quantikine ELISA Kit from R&D Systems (Minneapolis, MN) with a slope of 0.9-1.1 and an R<sup>2</sup> value > 0.9.

**Interleukin-1 receptor antagonist (IL-1ra):** IL-1ra is measured in serum by an enzyme-linked immunosorbent assay (ELISA) technique using the Human Interleukin 1 receptor antagonist Simple Plex Assay on the ELLA System from Protein Simple (San Jose, CA). The manufacturer inter-assay CV is 7.4% at a concentration of 27.2 pg/mL and 5.0% at a concentration of 1390 pg/mL. This assay has been correlated to the Quantikine ELISA Kit from R&D Systems (Minneapolis, MN) with a slope of 0.9-1.1 and an R<sup>2</sup> value > 0.9.

**Interleukin-6 (IL-6):** IL-6 is measured in serum by a enzyme-linked immunosorbent assay (ELISA) technique using the Human Interleukin 6 Simple Plex Assay on the ELLA System from Protein Simple (San Jose, CA). The manufacturer inter-assay CV is 8.3% at a concentration of 41.5 pg/mL and 7.1% at a concentration of 1800 pg/mL. This assay has been correlated to the Quantikine ELISA Kit from R&D Systems (Minneapolis, MN) with a slope of 0.9-1.1 and an R<sup>2</sup> value > 0.9.

**Interleukin-10 (IL-10):** IL-10 is measured in serum by a enzyme-linked immunosorbent assay (ELISA) technique using the Human Interleukin 10 Simple Plex Assay on the ELLA System from Protein Simple (San Jose, CA). The manufacturer inter-assay CV is 7.1% at a concentration of 33.2 pg/mL and 7.1 at a concentration of 1681 pg/mL. This assay has been correlated to the Quantikine ELISA Kit from R&D Systems (Minneapolis, MN) with a slope of 0.9-1.1 and an R<sup>2</sup> value > 0.9.

**Transforming growth factor beta 1 (TGF-β1):** TGF-β1 is measured in serum by a enzyme-linked immunosorbent assay (ELISA) technique using the Human Transforming Growth Factor beta 1 Simple Plex Assay on the ELLA System from Protein Simple (San Jose, CA). The manufacturer inter-assay CV is 6.9% at a concentration of 89.9 pg/mL and 6.9% at a concentration of 5109 pg/mL. This assay has been correlated to the Quantikine ELISA Kit from R&D Systems (Minneapolis, MN) with a slope of 0.9-1.1 and an R<sup>2</sup> value > 0.9.

**(soluble) Tumor Necrosis Factor (sTNFR-1):** sTNF R1 is measured in serum by a enzyme-linked immunosorbent assay (ELISA) technique using the Human Tumor Necrosis Factor Receptor 1 Simple Plex Assay on the ELLA System from Protein Simple (San Jose, CA). The manufacturer inter-assay CV is 11.8% at a concentration of 19.5 pg/mL and 10.0% at a concentration of 971 pg/mL. This assay has been correlated to the Quantikine ELISA Kit from R&D Systems (Minneapolis, MN) with a slope of 0.9-1.1 and an R<sup>2</sup> value > 0.9.

**Total Protein:** Total protein is measured in serum on a Roche Cobas 6000 Chemistry Analyzer (Roche Diagnostics Corporation) using a biuret reaction method (Roche Diagnostics, Indianapolis, IN). The laboratory inter-assay CV is 3.0%.

**Urea Nitrogen:** Urea nitrogen is measured in serum enzymatically using the Roche Urea/BUN reagent (Roche Diagnostics, Indianapolis, IN) on the Roche COBAS 6000 Chemistry analyzer (Roche Diagnostics Corporation). The laboratory inter-assay CV is 2.0% at a concentration of 15.3 mg/dL and 2.0% at a concentration of 54.0 mg/dL.

**Vitamin D (Vitamin D2 and D3):** Vitamin D is measured by LC/MS/MS. The internal standard, deuterated 25-hydroxyvitamin D3, deuterated 25-hydroxyvitamin D2 are added to serum samples. The analytes are extracted and precipitated from the specimen using n-heptane. Specimens are stored in -80 freezer for 2 hours. The organic layer is removed using a gasket apparatus and dried in a speed vacuum. The reconstituted specimens are injected into the LC/MS/MS. 25-hydroxyvitamin D2 and D3 and the internal standard, deuterated 25-hydroxyvitamin D3, are eluted off a Phenomenex column with a mobile phase of 71% 2.0 mmol/L ammonium acetate, in methanol 45°C then introduced via an APCI probe to monitor the compounds of interest. System control and data acquisition are performed by the Analyst software. MultiQuant software is employed for calibration and data processing. The laboratory is enrolled in the NIST Fat Soluble Vitamins Program.

**Assays performed on a subset of approximately 4000 HRS participants (Subsample assays)**

**Brain-derived neurotrophic factor (BDNF):** BDNF is measured in serum by an enzyme-linked immunosorbent assay (ELISA) technique using the Human Free Brain-Derived Neurotrophic Factor Simple Plex Assay on the ELLA System from Protein Simple (San Jose, CA). Manufacturer inter-assay CV is 10.5% at a concentration of 219 pg/mL and 10.2% at a concentration of 11,200 pg/mL. This assay has been correlated to the Quantikine ELISA Kit from R&D Systems (Minneapolis, MN) with a slope of 0.9-1.1 and an  $R^2$  value > 0.9.

**Clusterin/ApoJ:** Clusterin is measured in serum by enzyme-linked immunosorbent assay (ELISA) technique using the Human Clusterin Quantikine ELISA Kit from R&D Systems (Minneapolis, MN). The plates are measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA). The manufacturer Inter-assay CV is 7.2, 8.4, and 6.8% at mean levels of 22.1, 64.1, and 136 ng/ml.

**Homocysteine, total (tHcy):** Homocysteine is measured in serum using Liquid chromatography/tandem mass spectrometry (Water's 2795 Liquid Chromatogram equipped with an autosampler and column oven). Data is obtained using the MassLynx software with automated data processing by QuanLynx software. The laboratory inter-assay CV is 3.5, 4.2 and 3.7% at mean levels of 7.1, 13.0 and 24.8  $\mu\text{mol/L}$ .

**DNA methylation assay:** We are measuring whole genome DNA methylation markers using the MethylationEPIC BeadChip (Infinium) microarray (Illumina Inc.) that covers over 850,000 CpG methylation sites (850K). The 850K microarray contains >90% of the 450K sites, but adds 333,265 CpGs located in enhancer regions identified by the ENCODE and FANTOM5 projects. One microgram of DNA is undergoes bisulfite conversion using the EZ-96 DNA Methylation kit (Zymo Research Corp., CA, USA) following the manufacturer's recommendations for Infinium

assays. MethylationEPIC beadarray uses the Infinium HD chemistry Assay (Illumina Inc.) and uses the Infinium HD Methylation Assay Protocol (Illumina Inc.) for determination of methylation at all 850K sites.

**mtDNA copy number:** Quantitative real-time PCR-based method is used to measure relative mtDNA copy number. The ratio of mitochondria-specific gene (ND1) copy number to a nuclear gene (18s) copy number will be determined for each sample and standardized to a reference DNA sample to adjust for assay variability. The CV for this assay is 11%.

**RNA extraction:** Total RNA will be extracted from PAXgene tubes with the PAXgene Blood RNA Kit IVD from Qiagen Inc. (San Diego, CA). Extracted RNA is then stored at -80°C until further analysis.

**Telomere Length assay:** Telomere length is being measured using a real time PCR assay. The ratio of copies of telomere specific sequence to a nuclear gene (albumin) copy number will be determined for each sample and standardized to a reference DNA sample to adjust for assay variability. The CV for this assay is 8%.

**RNA Seq:** Total eukaryotic RNA isolates are quantified using a fluorimetric RiboGreen assay. Total RNA integrity is assessed using capillary electrophoresis (e.g., Agilent BioAnalyzer 2100), generating an RNA Integrity Number (RIN). Samples for which the RNA yield is  $\geq 1$  microgram and have an RIN of  $\geq 8$  or greater (the initial quality control screen) are converted to Illumina sequencing libraries. Total RNA samples are treated with the Globin-Zero Gold rRNA Removal Kit (Illumina Inc.) and are converted to Illumina sequencing libraries using Illumina's stranded mRNA Sample Preparation kit (Cat. # RS-122-2101). In summary, 1 microgram of total RNA is oligo-dT purified using oligo-dT coated magnetic beads, fragmented and then reverse transcribed into cDNA, fragmented, blunt-ended, and ligated to indexed (barcoded) adaptors and amplified using 15 cycles of PCR. Indexed libraries are then normalized, pooled and size selected to 320bp  $\pm$  5% using Caliper's XT instrument. TruSeq libraries are hybridized to a paired end flow cell and individual fragments are clonally amplified by bridge amplification on the Illumina cBot. Once clustering is complete, the flow cell is loaded on the HiSeq 2000 and sequenced using single end 50 base pair reads on Illumina's SBS chemistry. Each sample is sequenced to obtain ~40 million reads.

## Quality Control

### Shipping

92% of the samples arrived to the lab within 24 hours of collection and 99% within 48 hours. Our pilot work demonstrated that assays we examined are not affected if the blood is received within a 48 hour window (see Section on Pilot Studies).

**LABORATORY QUALITY CONTROL PROCEDURES:** The ARDL has standard quality control procedures to ensure integrity of stored samples and high quality of performance of laboratory procedures. All samples and records are maintained in secure areas. Samples are either stored in freezers that are within the laboratory which is locked whenever laboratory personnel are not present, or in freezers within locked rooms to which only study personnel and authorized maintenance personnel have access. Records are all stored within the laboratory. The freezers at the ARDL are monitored by local and remote sensor phone alarms, and a backup generator prevents long-term power outages. An electronic inventory management system permits the laboratory to track specimen receipt, enter data, import and translate data, track and resolve discrepancies, prepare reports and manage freezer inventory space. Besides inventory records, the system tracks processes performed on a specimen such as shipping, return to inventory, aliquoting, thawing and facilitates sample retrieval for laboratory testing and ancillary studies. In addition, internal quality assurance procedures are routinely performed in the ARDL and are monitored on a weekly basis for this study. Internal quality assurance is maintained by the following procedures: (1) routine calibration of the instruments with standards, (2) analysis of control pools with each analytical run, (3) continual monitoring of control pool values, response factors and internal standard recoveries for all analytes. Thus, all assays have at least the following quality assurance procedures:

1. The results from the control pool analysis are used as checks for trends, equipment failures, and possible preparation problems.
2. Levy-Jennings charts are maintained to determine assay consistency.
3. The control values are evaluated by Westgard rules, when applicable. Any batches that do not pass the Westgard rules or are outside the normal physiological range are reanalyzed and reevaluated for the study.
4. The same quality control policies are used to evaluate each batch analysis. Two control pools are established for each assay, with values that are in the physiological range (one having a relatively high value and the other a relatively low value).
5. Assay sensitivity, specificity, linearity, analyte recovery, limit of detection, accuracy and precision are evaluated for each method.
6. Blinded quality control samples, monitored by an independent statistician, are included with study subject samples. These data are analyzed for assay precision and provide an independent measure of quality assurance. If the matched pairs of samples do not agree with each other, remedial action will be taken to reestablish assay performance.

*Quality control for Sample Shipment:* Staff have been informed of when to expect shipments and trained in follow-up procedures for any late-arriving shipments so that samples are prepared in the shortest time frame possible.

The ARDL is compliant with CFR21 Part 11 and will report all laboratory results daily using a file transfer protocol (FTP). Any tests whose results exceed their alert range will be flagged appropriately.

**Laboratory procedures for assay specific quality control:** *Accuracy of analyte measurement.* To ensure accuracy of reported laboratory values obtained in the HRS, all assays are calibrated using standard reference material (SRM) from the National Institute of Standards and Technology (NIST) or NIST traceable SRMs when available (e.g. cholesterol, HDL, creatinine and HbA1C). For analytes without NIST or NIST traceable SRMs, the lab monitors accuracy by regular participation in external proficiency programs. Details on the equipment used and general laboratory inter-assay CV are shown in the description of each assay. Calculations made from special quality control samples are shown below.

### Duplicate Quality Control Samples

Duplicate control samples (n=204), collected from age appropriate volunteers. These were barcoded and packaged using kits assembled by Hooper Holmes so as to be indistinguishable from participant samples to the lab and then shipped from the University of Michigan to serve as an additional quality control. These duplicate controls were assayed with study samples and used for assessing reliability for many of the assays. Extra material from these samples is now stored to be used in the future to determine future assay reliability and comparability.

Results comparing the assay pairs from the 190 to 204 usable duplicate samples are shown below. Comparability for most of the pairs is extremely high with modal reliability at .99. Only 4 of the assays have a coefficient of variation that is above 10%:

	Quality Control Measures from Assay Pairs					
	N	Mean(6)	Within SD (Lab) (1)	Between SD (2)	Reliab (3)	CV (4)
<b>WBC</b>	190	6.4	1.88	1.88	0.9918	1.9
<b>RBC</b>	190	4.65	0.42	0.42	0.9919	0.7
<b>HGB</b>	190	14.13	1.3	1.3	0.9929	0.6
<b>HCT</b>	190	42.59	3.44	3.45	0.988	0.7
<b>MCV</b>	190	91.87	4.55	4.56	0.9889	0.3
<b>MCH</b>	190	30.46	1.83	1.83	0.99	0.5
<b>MCHC</b>	190	33.17	0.96	0.97	0.9454	0.5
<b>RDW</b>	190	13.18	1	1	0.9907	0.5
<b>PLT</b>	188	244.12	53.69	53.83	0.9808	2.1
<b>MPV</b>	186	11.09	1	1.01	0.9805	0.9
<b>PDW</b>	186	13.56	2.26	2.27	0.9362	2.8
<b>NEUT</b>	190	60	9.2	9.22	0.9924	1.1
<b>LYMP</b>	190	29.2	8.26	8.28	0.9926	2.1
<b>MONO</b>	190	7.58	1.7	1.7	0.9133	5.4
<b>EOS</b>	190	2.59	1.53	1.53	0.9736	8.8
<b>BASO</b>	190	0.64	0.36	0.36	0.7518	19.8
<b>ANEU</b>	190	3.9	1.61	1.61	0.9949	2.1
<b>ALYM</b>	190	1.81	0.56	0.56	0.9828	3.1
<b>AMON</b>	190	0.47	0.14	0.14	0.9045	6.2
<b>AEOS</b>	190	0.16	0.1	0.1	0.8968	12.4
<b>ABAS</b>	190	0.03	0.05	0.05	0.6334	57.3

ALT	204	21.26	13.07	13.1	0.9981	2.3
ALB	204	4.18	0.31	0.31	0.9216	1.5
ALK P2	204	80.7	21.31	21.36	0.9957	1.5
AST	204	22.66	8.64	8.66	0.9953	1.8
BILT	204	0.41	0.19	0.19	0.963	5.1
BUN	204	14.54	4.07	4.08	0.9928	1.3
CA	204	9.39	0.41	0.41	0.9594	0.7
LDLC	204	108.29	30.56	30.63	0.9932	2.1
CL	204	100.62	2.32	2.32	0.9437	0.3
CHOL	204	195	38.54	38.63	0.9919	1.5
CO2	204	23.6	2.04	2.04	0.8506	2.3
CR	204	0.83	0.22	0.22	0.9958	1.4
CRP	204	2.31	5.07	5.08	0.9999	2
CYSC	204	0.91	0.31	0.31	0.9909	2.7
FERTN	202	87.4	80	80.2	0.9992	2.2
GLUFF	204	98.79	39.22	39.32	0.9975	1.5
HDL	204	64.32	20.15	20.19	0.9961	1.3
K	204	4.14	0.26	0.26	0.8885	1.3
NA	204	137.97	2.38	2.39	0.9442	0.3
TP	204	7.04	0.43	0.43	0.9242	1.2
TGF	204	112.68	53.84	53.97	0.9981	1.6
CMVGE	204	172.35	309.69	310.45	0.988	11.1
DHEASE	204	4.33	2.79	2.8	0.9972	2.6
NTBNPE	190	77.84	136.08	136.41	0.9989	2.8

## Pilot Studies Assessing Validity and Reliability of Methods

### Pilot 1: Basic design was pre-tested during the 2010 wave of HRS

The basic design of this study was pre-tested during the 2010 wave of HRS. We selected a random sample of about 200 households in diverse areas of the country. The request to permit future contact by a phlebotomist to draw a whole blood sample was made at the end of the 2010 interview, so the request could not affect 2010 interview response. They could decline at that point and, in the pilot experiment, no further attempt at persuasion was made. They could also decline to set an appointment when contacted by the phlebotomy service and they could either miss the appointment (at their home) or decline to sign the consent form or to provide a sample at the time of the appointment. The final completion rate was 69.3%.

An important issue for a longitudinal study like the HRS is the impact of additional requests on future participation in the core survey. All major increments to HRS have been tested on small samples for response effects before integration to the full sample. In 2012 the interview rate for persons who were asked to provide a blood sample was 95.9%, compared with 95.4% for those in the eligible control group who also gave a 2010 interview but were not selected to be asked. There is thus no evidence of a harmful effect on longitudinal participation.

Also in the 2010 pilot, we performed a limited test of the quality of the blood draw itself. Phlebotomists collected five tubes of blood and shipped them overnight to a central lab for processing and storage at -80C. The properties of serum and plasma collected and processed with a 24 to 48 hour overnight shipping delay are well known and the limitations of this mode of collection are fully taken into account in our planning for assays to be done. We particularly wanted to test the quality of the RNA extracted from the PAXgene tubes. We contracted with Dr. Steven Cole of UCLA to analyze our pilot PAXgene samples. He found that 121 out of 122 samples had large amounts of detectable RNA, many fold greater than needed for microarray analysis. The RNA also exceeded necessary levels of purity. From the pilot samples, Cole initially assayed 6 RNA markers: *ACTB* (a housekeeping/ normalization marker); *IL1B*, *IL8*, *PTGS2* (proinflammatory markers); *SOD2* (oxidative stress); *IFI27* (Type I interferon). In duplicate RT PCR assays of all 121 samples there was strong consistency of results; test-retest correlations averaged .87 (range .73-.96). These pilot results indicated that the PAXgene tubes provided good quality, reliable samples for assay. The PAXgene samples were then used to pilot the use of a transcriptome approach.

Several publications resulted from this pilot work:

Contemporaneous Social Environment and the Architecture of Late-Life Gene Expression Profiles. Morgan Levine, Eileen Crimmins, David Weir, Steve Cole. [American Journal of Epidemiology](#). 186:503-509. PMID: 28911009

Loneliness, Eudaimonia, and the Human Conserved Transcriptional Response to Adversity. 2015. S.W. Cole, M.E. Levine, J. M. G. Arevalo, J. Ma, D.R. Weir, E.M. Crimmins. [Psychoneuroendocrinology](#), 62: 11-17. PMID: 26246388; PMC4637182.

Childhood and Later Life Stressors and Increased Inflammatory Gene Expression at Older Ages. 2015. Morgan Levine, Steven Cole, David Weir, Eileen Crimmins. [Social Science and Medicine](#), 130:16-22. PMID: 25658624; NIHMSID 672793.

### **Pilot 2: Second pilot during the 2014 HRS pretest using the proposed collection and shipping protocol**

We performed a second pilot during the 2014 HRS pretest using the exact proposed collection and shipping protocol to test our ability to get samples to the lab and conduct flow-cytometry within 1-2 days. From a range of geographic areas, 20 cases were selected to have the proposed blood draw and 14 were completed. Samples were collected, centrifuged where needed and shipped refrigerated to the University of Minnesota. All samples arrived within 24 hours and were evaluated for T cell subsets (CD4+ and CD8+), B cells, Natural Killer (NK) cell subsets and monocyte subsets. All results were within the expected range of values for the various cell subsets. Independent stability studies of flow cytometry measurements at the University of Minnesota showed that all measurements were stable for up to 72 hours.

### **Pilot 3: Clarify the effect of delayed processing and cryopreservation on immune cell subsets measured in flow cytometry**

Healthy volunteers (N=20) provided 8 tubes of blood which were collected into 2 types of tubes, CPT and EDTA. The CPT tubes were processed and lymphocytes cryopreserved at 4 different time points: immediately (<90 minutes), after 24 hours, after 48 hours, after 72 hours. The EDTA tubes were processed after the same delays. The point of the pilot was to

- Evaluate the effect of delayed cell processing on immune cell subsets.
- Evaluate the effect of cryopreservation on immune cell subsets.
- Evaluate the effect of cryopreservation at various time points on immune cells subsets.

The results indicated that delay in processing for up to 72 hours has very little effect on most cell subsets that we are measuring. Cryopreservation significantly affects the quantification of B cells. T cells and all the measured subsets as well as dendritic cells can be reliably measured in cryopreserved samples up to 48 hours post blood draw. At 72 hours, while the populations remain highly correlated. B cells, NK cells and monocytes are significantly affected by delayed processing and cryopreservation. These populations remained strongly correlated throughout the time points ( $r \geq 0.61$ ). This suggests that time interval between sample collected and cryopreservation can be used as a covariate during the data analysis for analysis of immune cell subsets in HRS

The results of this pilot were presented as a poster titled "Effect of cryopreservation on multiparameter flow cytometry: Implications for the Health and Retirement Study." Hélène Barcelo, Sharon Minnerath, David Weir, Eileen Crimmins, Jessica Faul, Bharat Thyagarajan at the International Society for Advancement of Cytometry's (ISAC) 32<sup>nd</sup> International Congress (CYTO 2017) held June 10-14 in Boston, Massachusetts.



## **Early Assay Results**

Below we compare the HRS assay results to those in the NHANES 57+ sample in order to assess the similarity of the values and distributions. Here we show the comparison based on the final 9,934 cases. For this comparison we use preliminary weights (vbswt2) for the HRS sample which eliminates 709 people who are not in the sample age range or are age ineligible. In addition, 36 other cases are eliminated as they do not have data in the preliminary release of the Core data. We provide descriptive statistics as well as distributions for the HRS sample and the NHANES 57+ sample. In general, the two studies have results that are quite similar, although statistically different, for a majority of the assays. Some differences between the two studies are expected given the differences in collection procedures, laboratory equipment and assays as well as the initial shipping and handling of the samples. The significant differences in the hematological parameters (especially the mean platelet volume) estimated by the complete blood count in both studies likely reflects the 24-48 hour delay in measuring complete blood counts at the central laboratory in HRS.

**Comparison of Assays: HRS 2016 VBS Age Eligible Sample Compared to Age 57+ Sample for NHANES**

	HRS 2016 VBS					NHANES 2011-2014				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
White Blood Cell Count	8880	6.74	2.83	1.60	209.00	3756	6.98	2.27	2.30	55.70
Red Blood Cell Count	8895	4.50	0.51	2.08	7.16	3756	4.50	0.50	1.67	8.30
Hemoglobin	8897	13.68	1.55	5.20	21.10	3756	13.97	1.47	6.40	18.80
Hematocrit	8892	41.78	4.28	19.30	58.50	3756	40.99	4.15	17.90	55.40
Mean Corpuscular Volume	8892	93.21	6.16	59.00	126.00	3756	91.34	5.36	58.40	115.30
Mean Corpuscular Hemoglobin	8874	30.51	2.04	20.00	39.50	3756	31.12	2.32	18.30	74.50
Mean Corpuscular Hemoglobin Concentration	8806	32.77	1.24	29.00	37.50	3756	34.06	1.19	29.20	69.60
Red Cell Distribution Width	8894	13.95	1.43	10.90	39.30	3756	13.47	1.17	11.00	25.90
Platelet Count	8881	230.84	65.03	8.00	916.00	3756	224.70	61.81	18.00	681.00
Mean Platelet Volume (MPV)	8783	11.20	1.01	8.10	15.20	3756	8.39	0.96	5.60	13.50
Platelet Distribution Width (PDW)	8780	13.85	2.38	8.30	25.50					
% Neutrophils	8876	57.99	9.59	0.00	100.00	3750	59.74	9.97	0.80	96.60
% Lymphocytes	8877	29.39	8.85	3.80	98.00	3750	28.13	8.82	2.70	88.00
% Monocytes	8877	8.56	2.41	0.00	43.50	3750	8.40	3.16	0.60	66.90
% Eosinophils	8877	3.19	2.22	0.00	33.70	3750	3.02	2.21	0.00	36.60
% Basophils	8876	0.90	0.64	0.00	10.60	3750	0.76	0.51	0.00	7.50
Neutrophil Count	8876	3.95	1.53	0.00	22.80	3750	4.22	1.67	0.10	15.00
Lymphocyte Count	8877	1.96	2.15	0.40	201.10	3750	1.93	1.17	0.20	49.00
Monocyte Count	8877	0.57	0.23	0.00	78.50	3750	0.57	0.27	0.10	10.20
Eosinophil Count	8877	0.21	0.17	0.00	4.90	3750	0.21	0.18	0.00	3.70
Basophil Count	8876	0.06	0.06	0.00	1.00	3750	0.05	0.06	0.00	0.80
Alanine Aminotransferase	9098	21.10	13.80	3.00	313.00	3648	22.81	13.15	5.00	228.00
Albumin	9110	3.96	0.33	2.20	5.50	3651	4.22	0.31	2.10	5.40
Alkaline phosphatase	9106	81.93	33.54	20.00	1908.00	3650	68.55	27.31	14.00	729.00
Aspartate Aminotransferase	9030	22.53	11.66	6.00	283.00	3647	25.57	12.59	9.00	220.00
Bilirubin, Total	9095	0.47	0.26	0.10	3.30	3648	0.68	0.28	0.10	4.10
Urea Nitrogen (BUN)	9110	17.87	7.01	3.00	99.00	3650	16.07	7.15	1.00	95.00
Calcium	9098	9.32	0.40	3.20	11.50	3642	9.44	0.38	6.50	12.00
LDL-Cholesterol, Calculated	8926	102.92	35.63	5.00	325.00	1655	112.88	39.14	14.00	331.00
Chloride	9109	100.88	3.17	82.00	114.00	3650	103.64	3.24	70.00	119.00
Cholesterol, Total	9109	189.34	42.25	55.00	542.00	3666	194.05	43.51	75.00	525.00
Bicarbonate (CO2)	9095	24.07	2.58	15.00	43.00	3650	25.62	2.38	16.00	43.00

Creatinine	9119	0.95	0.47	0.28	14.10	3651	0.98	0.61	0.40	17.41
C-Reactive Protein (high sensitivity) <sup>1</sup>	9107	4.73	11.69	0.21	362.70	3957	4.16	8.48	0.10	200.00
Cystatin C <sup>2</sup>	9109	1.17	0.51	0.36	9.22	3034	1.08	0.48	0.43	10.70
Ferritin <sup>3</sup>	9104	154.17	241.48	5.00	8231.00					
Glucose, Fasting	9075	110.04	42.02	26.00	574.00	1701	112.07	34.25	47.00	405.00
HDL-Cholesterol, Direct-Measure	9110	57.61	19.58	11.00	187.00	3666	55.09	17.52	16.00	173.00
Potassium	9090	4.21	0.42	2.40	8.80	3649	4.06	0.40	2.30	7.30
Sodium	9109	139.44	2.86	117.00	156.00	3650	139.52	2.70	102.00	148.00
Protein, Total	9110	6.87	0.46	4.70	10.10	3646	6.96	0.48	5.20	10.20
Triglycerides	9109	146.57	94.10	30.00	1754.00	1667	135.32	107.13	29.00	1380.00
CMV IgG <sup>4</sup>	9107	303.99	392.29	0.10	22801.00					
%CMV IgG Non-Reactive/Negative	2567	33.92%				2218	42.19%			
Reactive/Positive	6419	64.73%					57.81%			
Borderline	121	1.34%					0.00%			
Dehydroepiandrosterone Sulfate (DHEAS)	9085	2.33	1.83	0.01	18.17	1214 <sup>a</sup>	3.34	0.33		
B-type natriuretic peptide, N-terminal pro (NT-proBNP)	8958	332.59	1210.66	5.00	69730.00	2975 <sup>b</sup>	111.7 (median)		5	35000
Logged NT-proBNP	8958	4.78	1.30	1.61	11.15					

NHANES 2011-2014: weight applied; HRS 2016 vbs: bioweight (vbsw2) applied.

HRS Age is from early release of Core data.

HRS LDL, glucose and triglycerides do include non-fasting sample.

Fasting weight is applied to Glucose, LDL and triglycerides in NHANES.

<sup>1</sup>NHANES CRP comes from 2007-2010.

<sup>2</sup>NHANES Cystatin C comes from 1999-2002 (Surplus sera cystatin 99-02 weights applied).

<sup>3</sup>NHANES Ferritin is available only for those under 50.

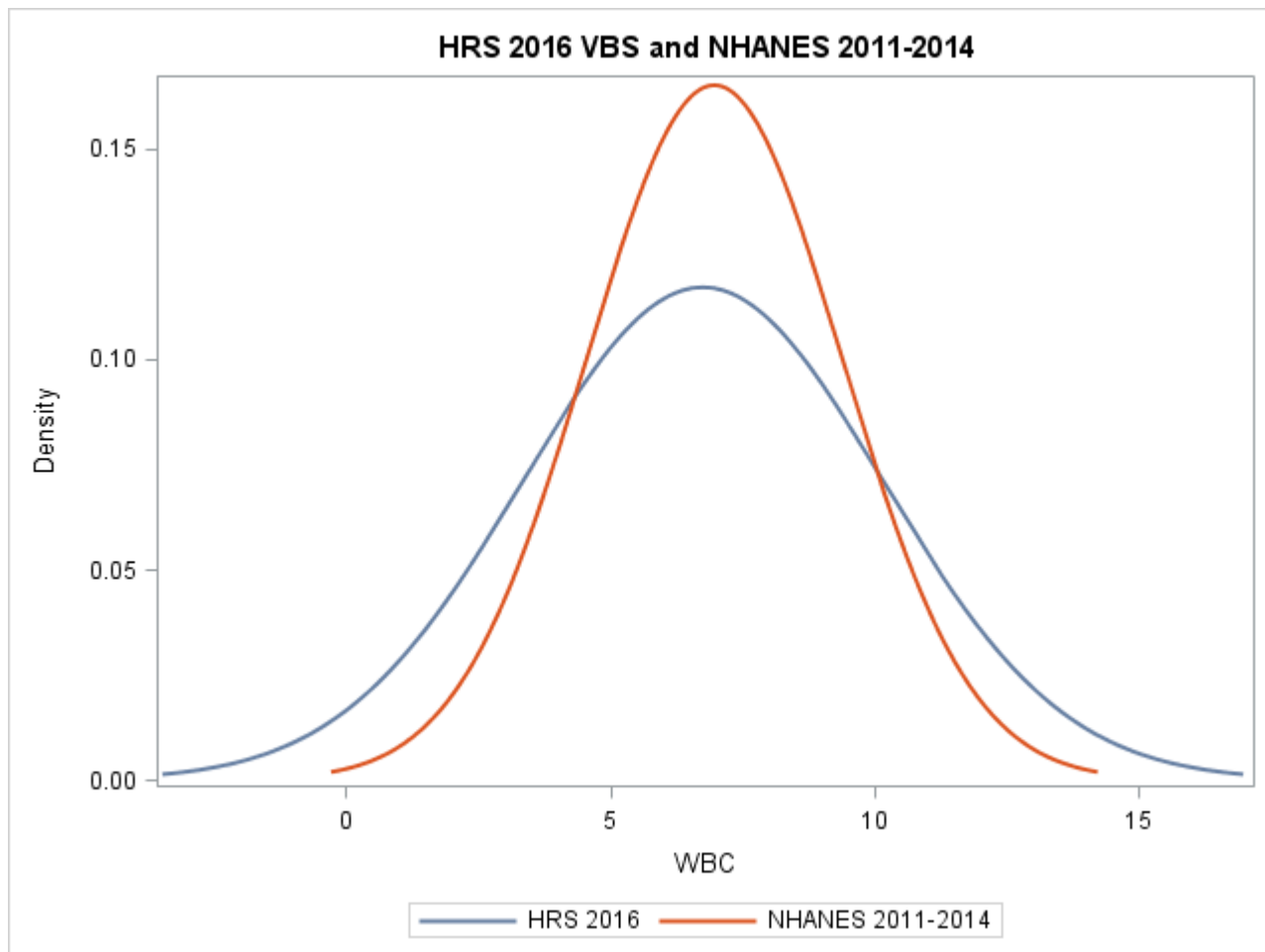
<sup>4</sup>CMV IgG: has only positive, negative indeterminate in NHANES 1999-2004 surplus, for 6-49 years old. In this table those 40-49 years old are reported.

<sup>a</sup>Mean of DHEAS is from MIDUS sample, age 35-86. Computed from Redina et al. 2016

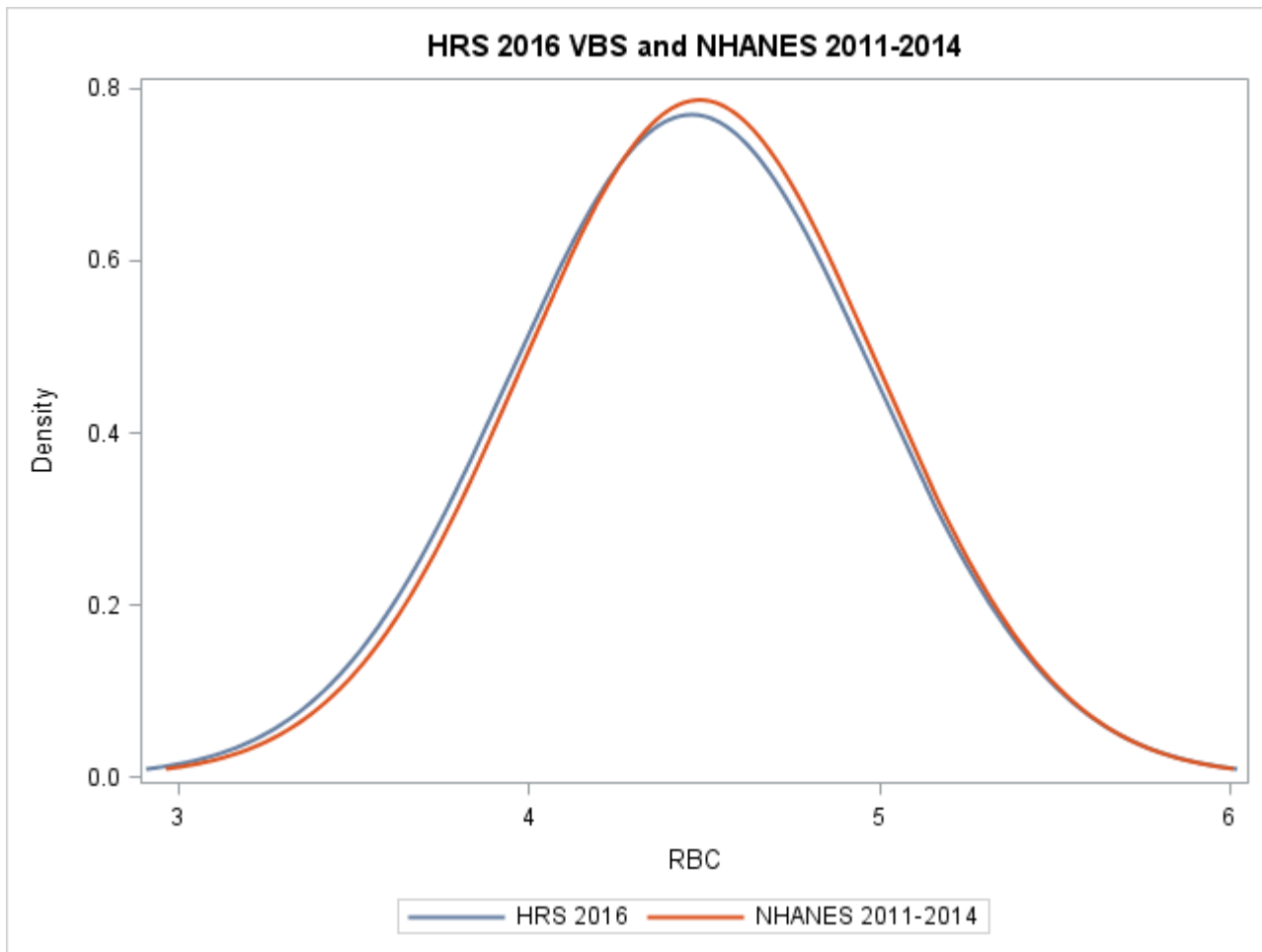
<sup>b</sup>Mean of NT-proBNP is from Cardiovascular Health Study sample, age 65+, from deFilippi et al. 2010 (in Journal of the American College of Cardiology).



The Following Density Plots do not have a weight applied  
White Blood Cell Count

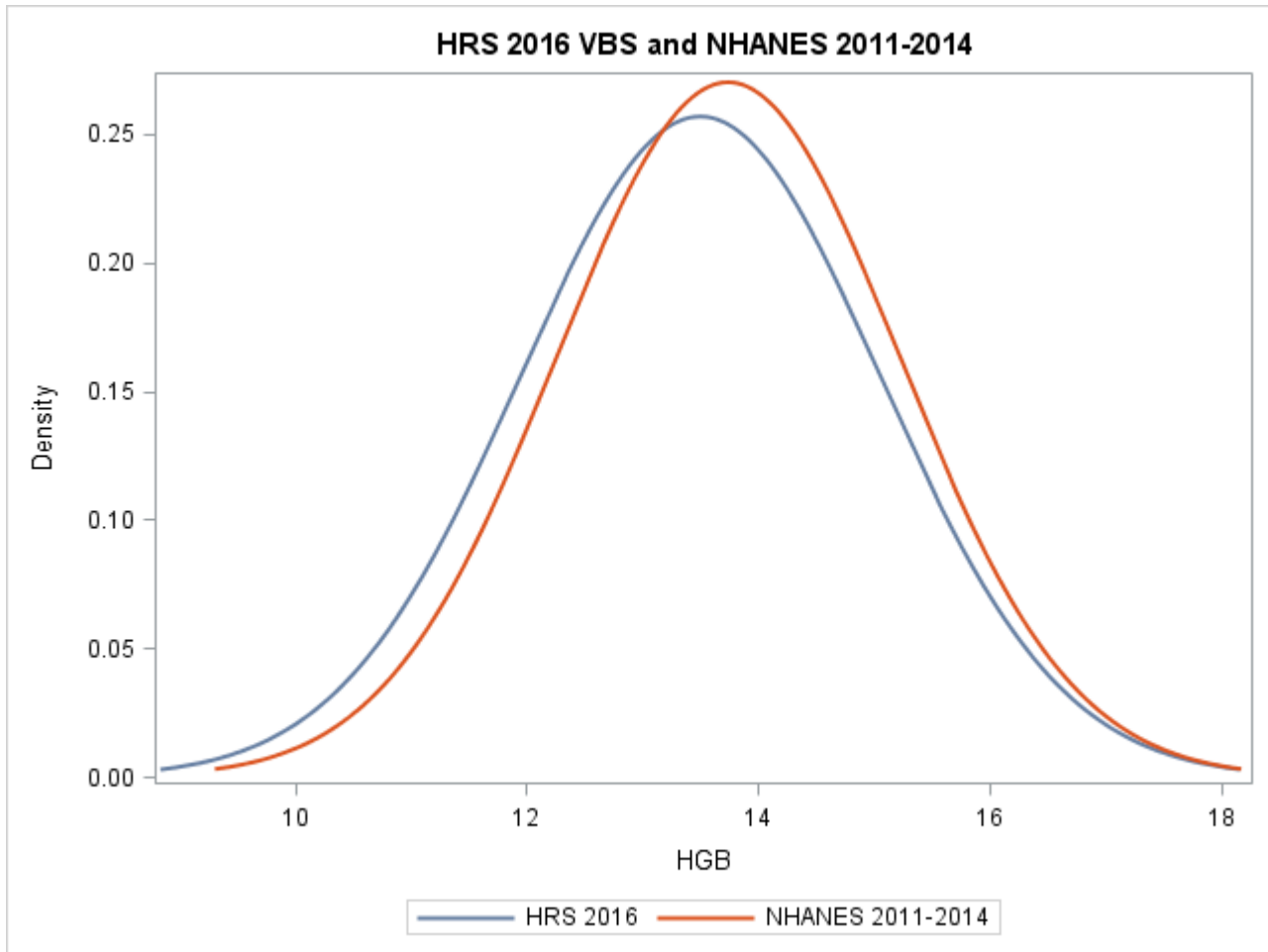


## Red Blood Cell Count



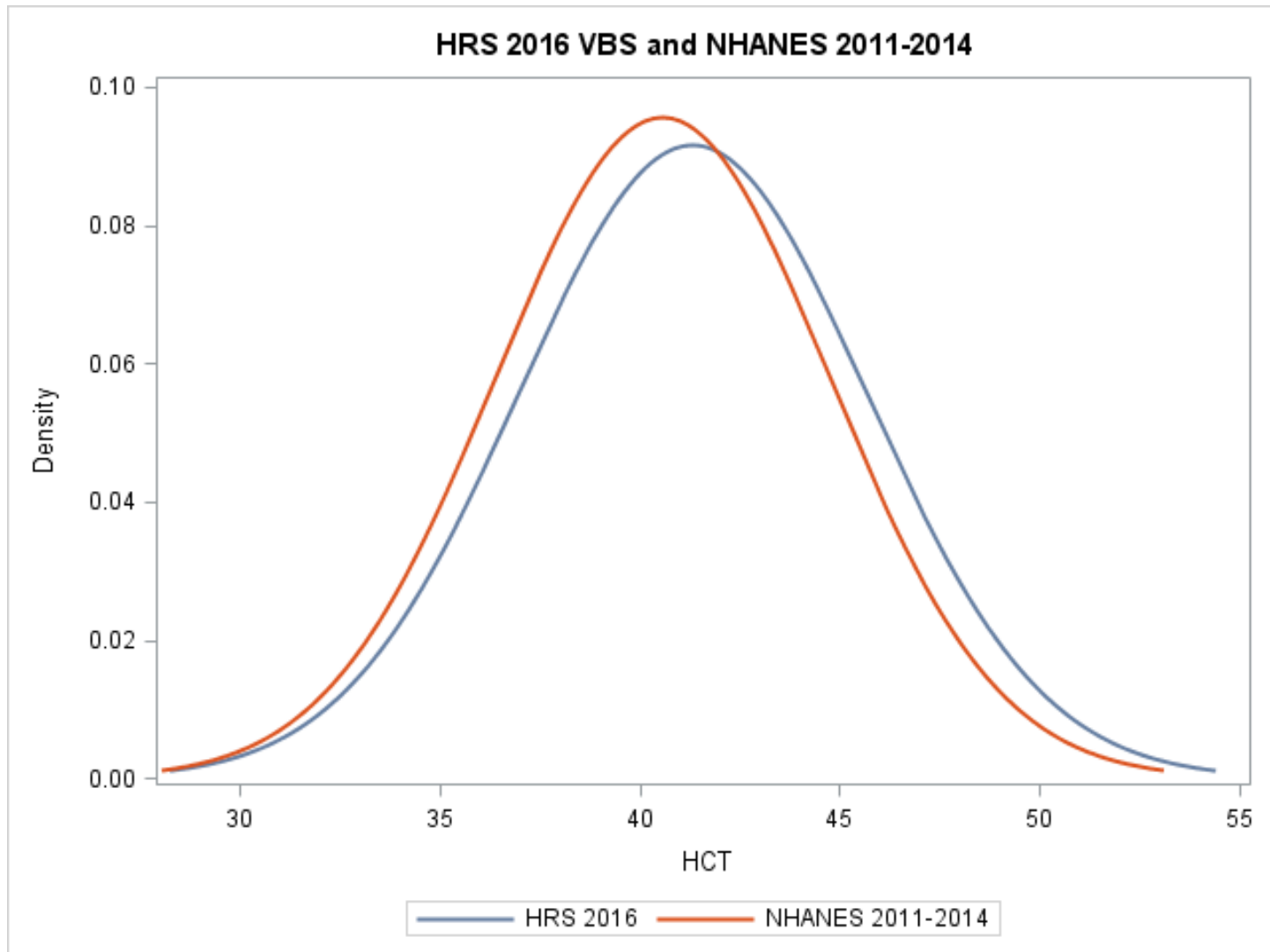


## Hemoglobin

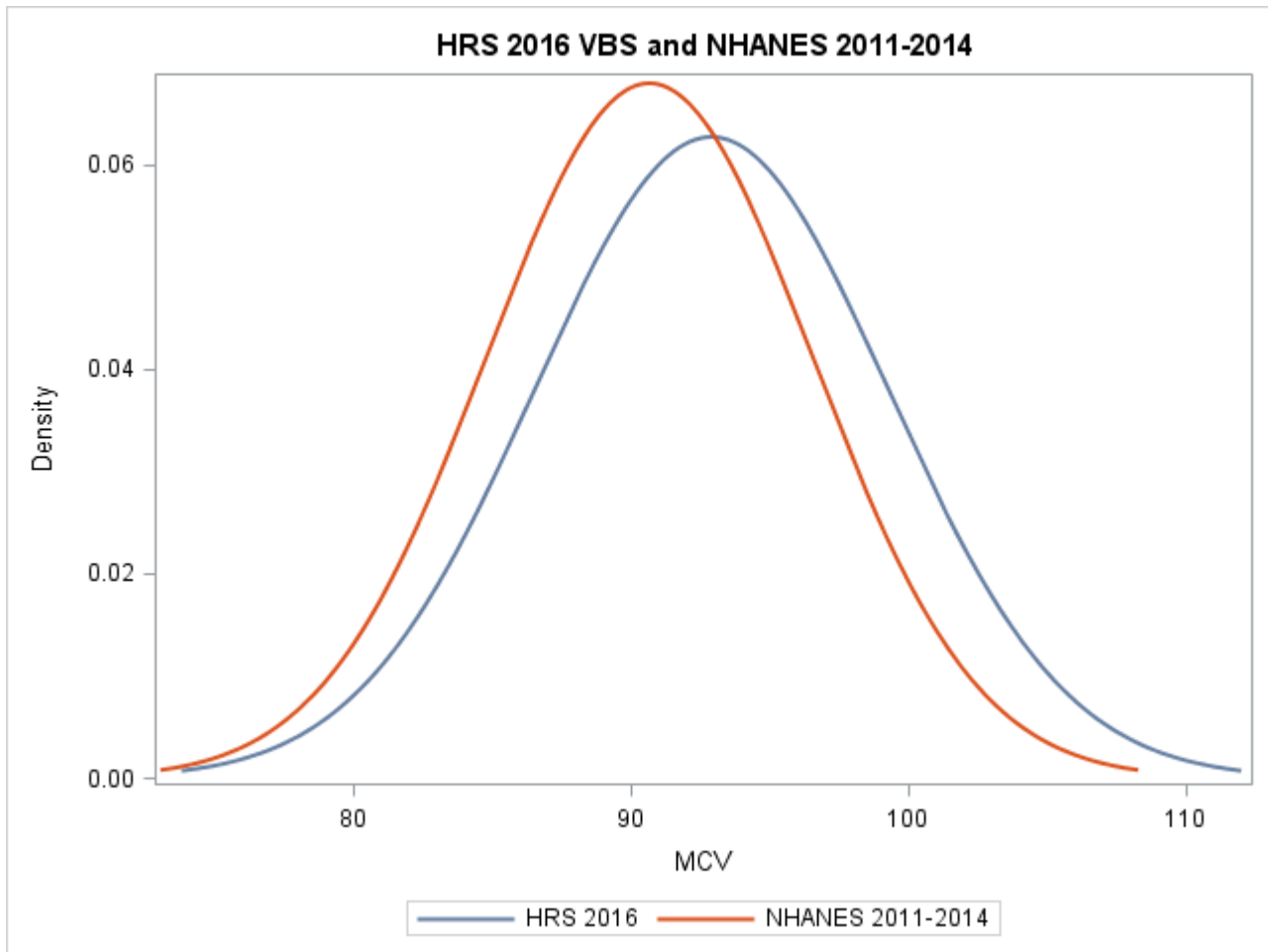




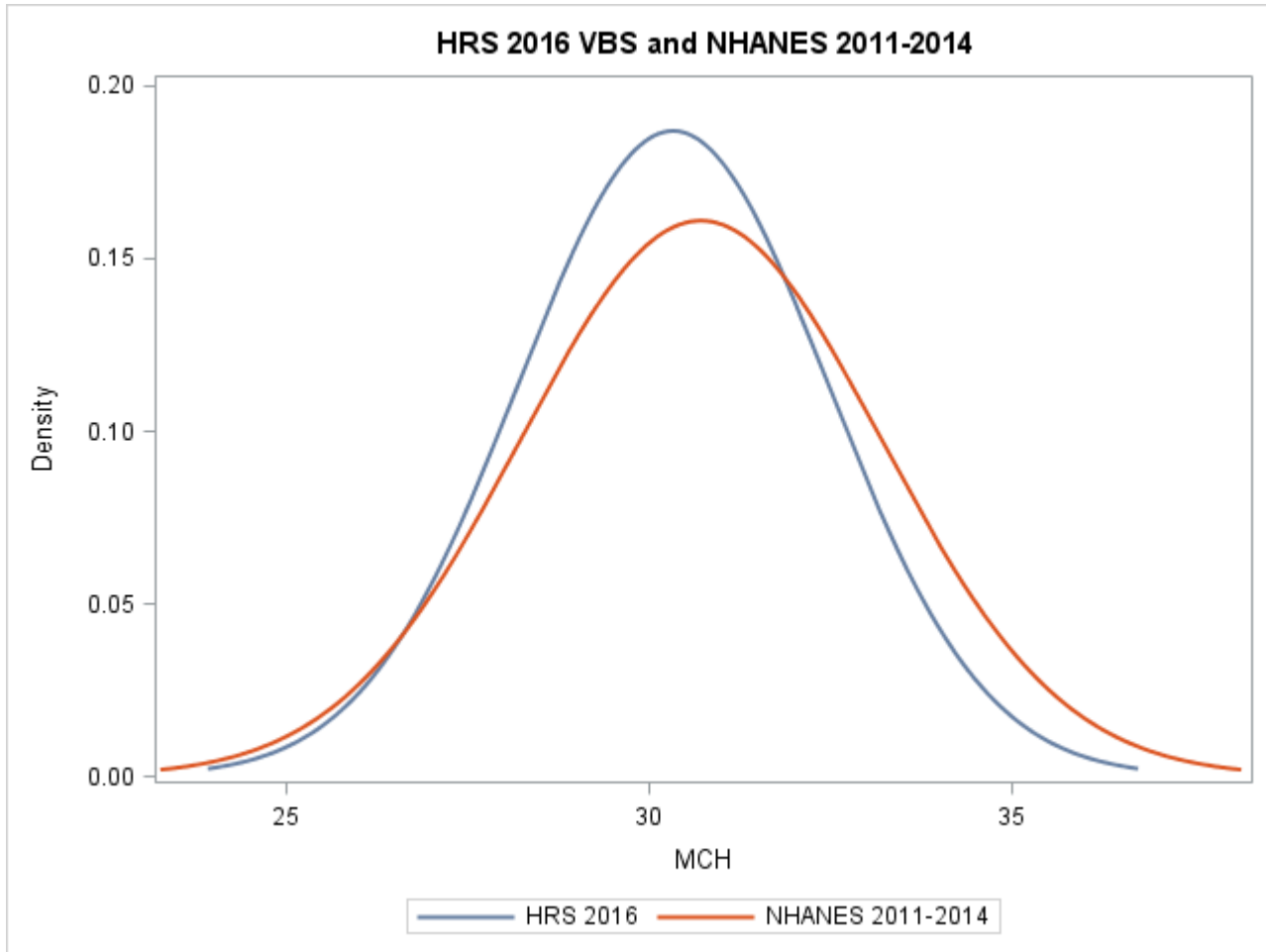
# Hematocrit



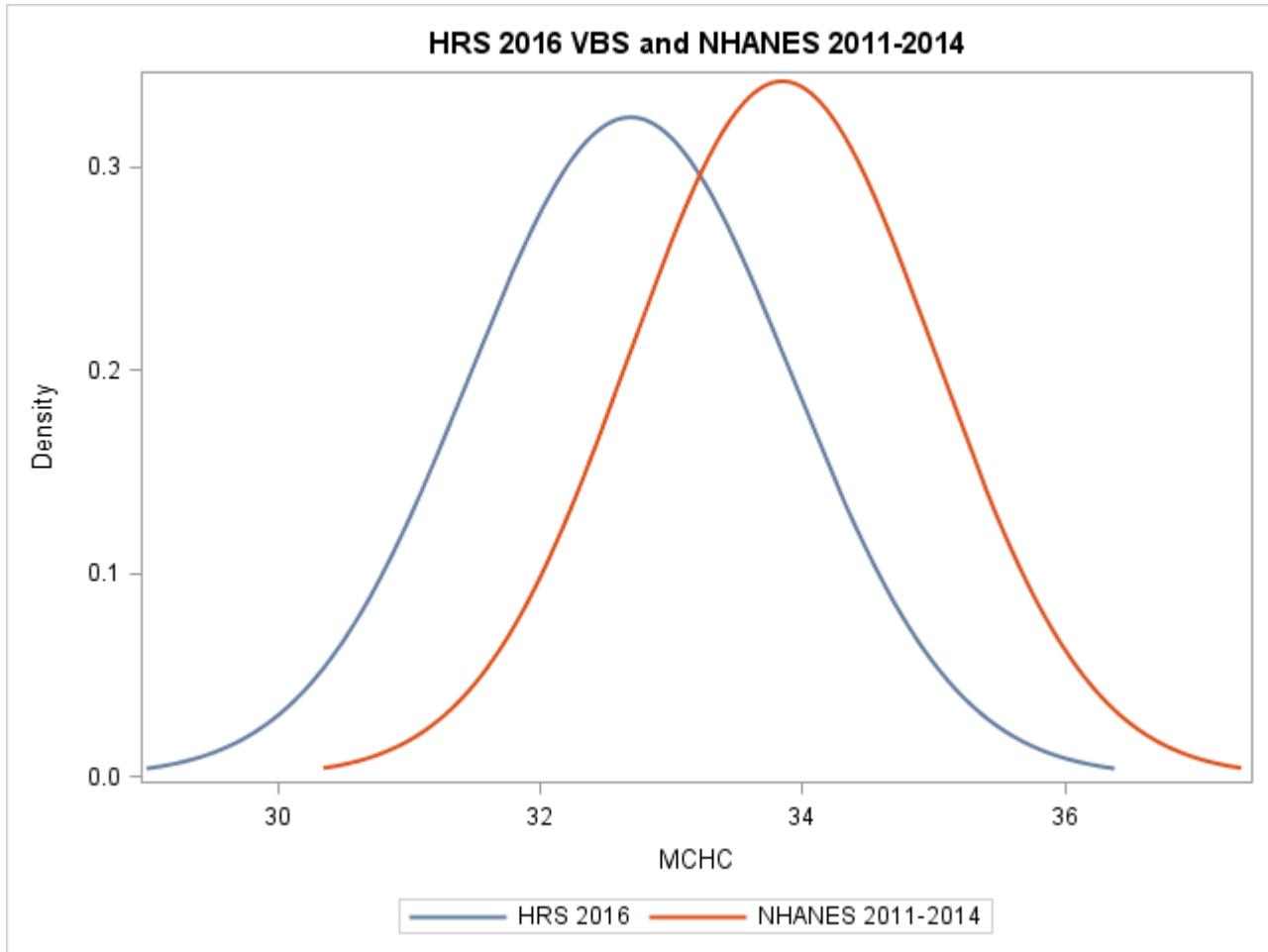
## Mean Corpuscular Volume



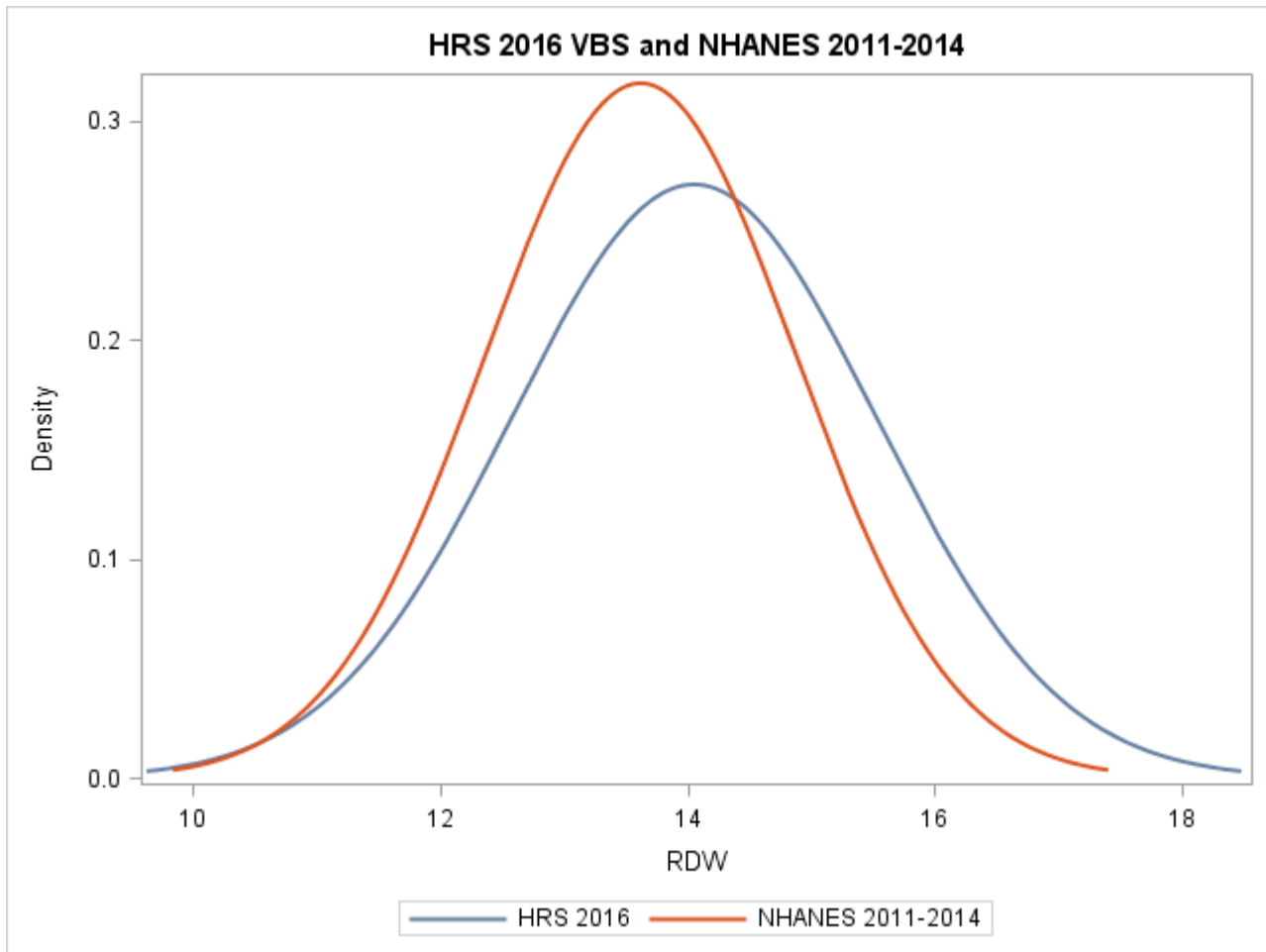
## Mean Corpuscular Hemoglobin



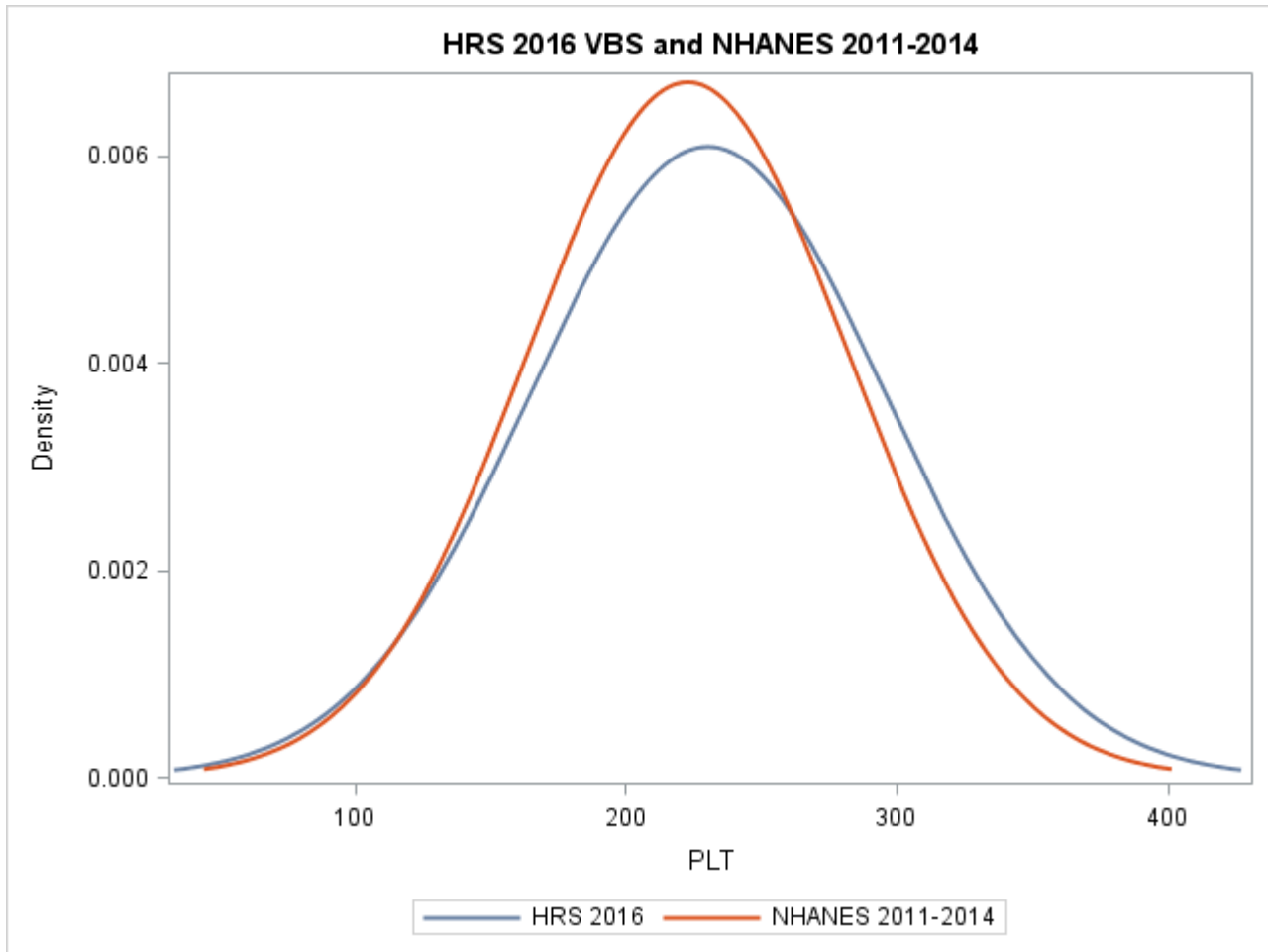
## Mean Corpuscular Hemoglobin Concentration



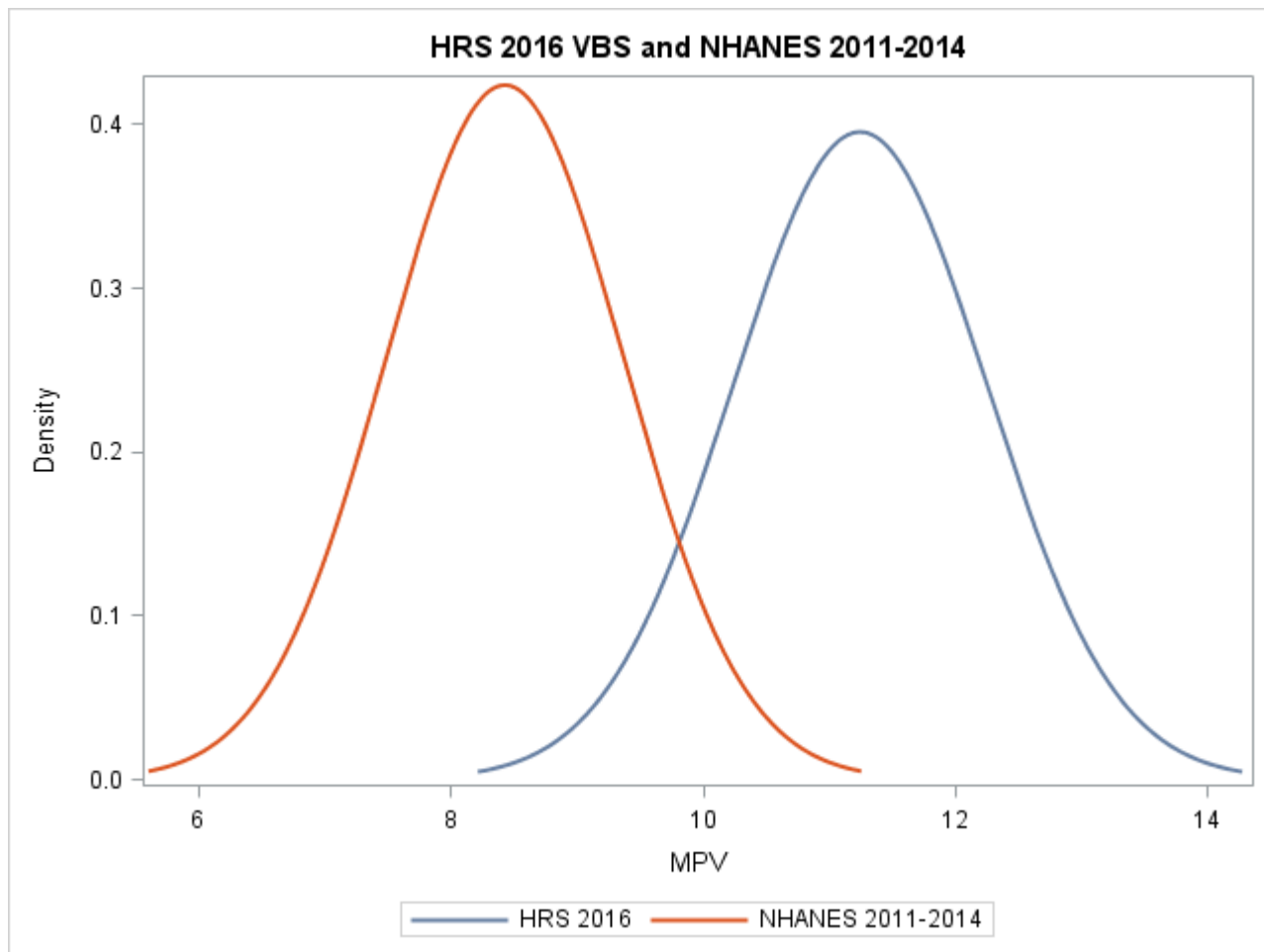
## Red Cell Distribution Width



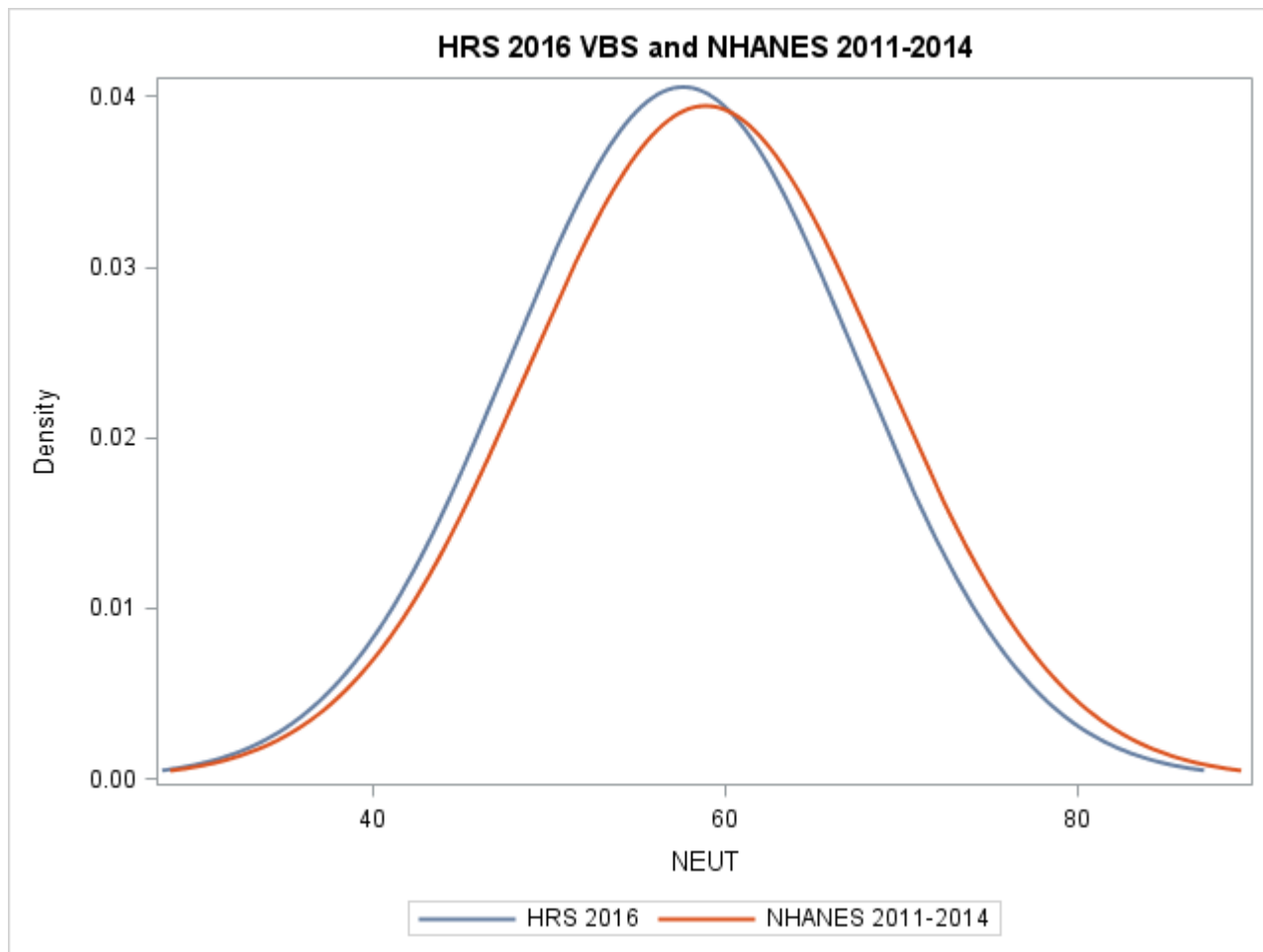
## Platelet Count



### Mean Platelet Volume

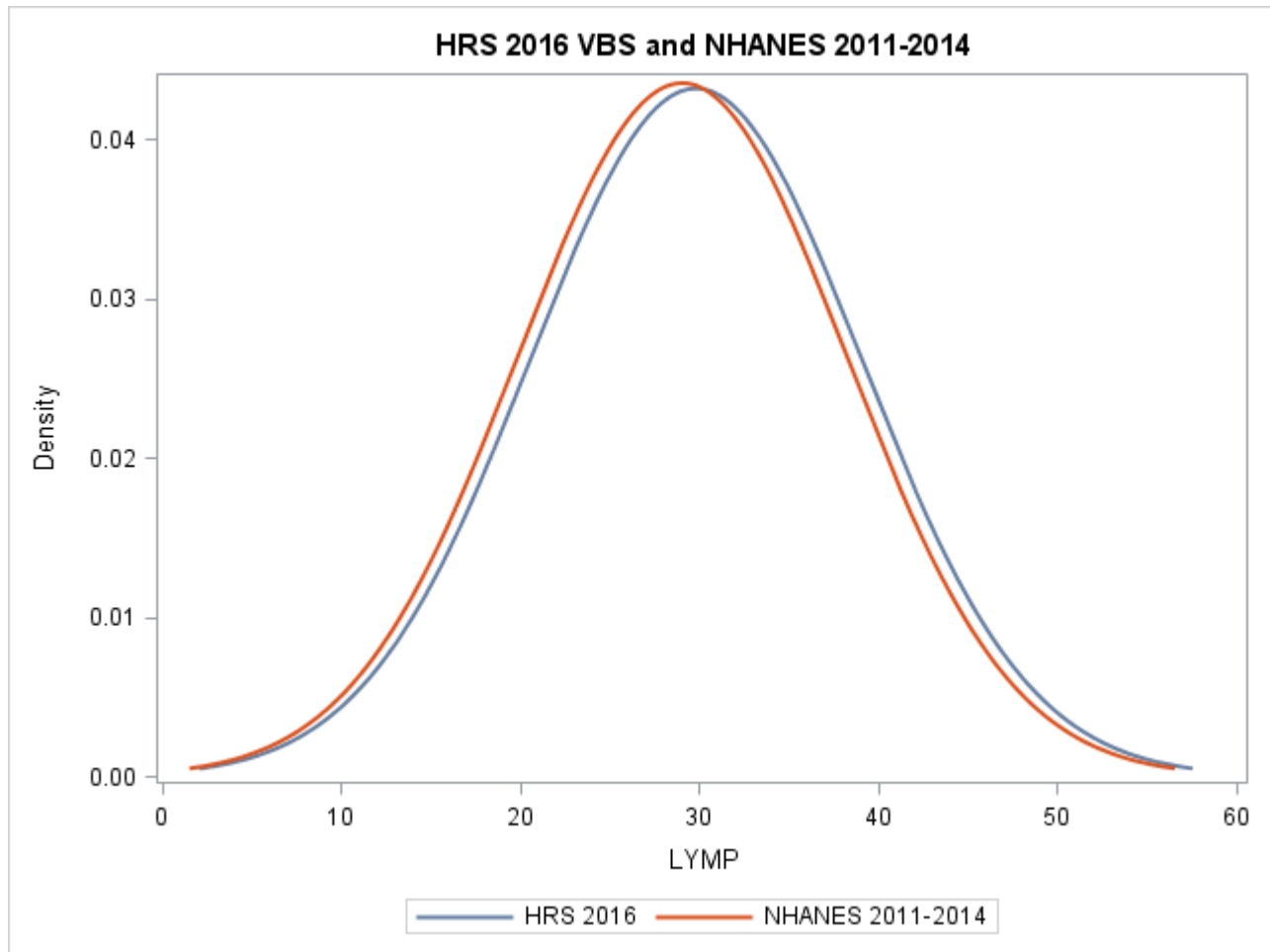


# % Neutrophils

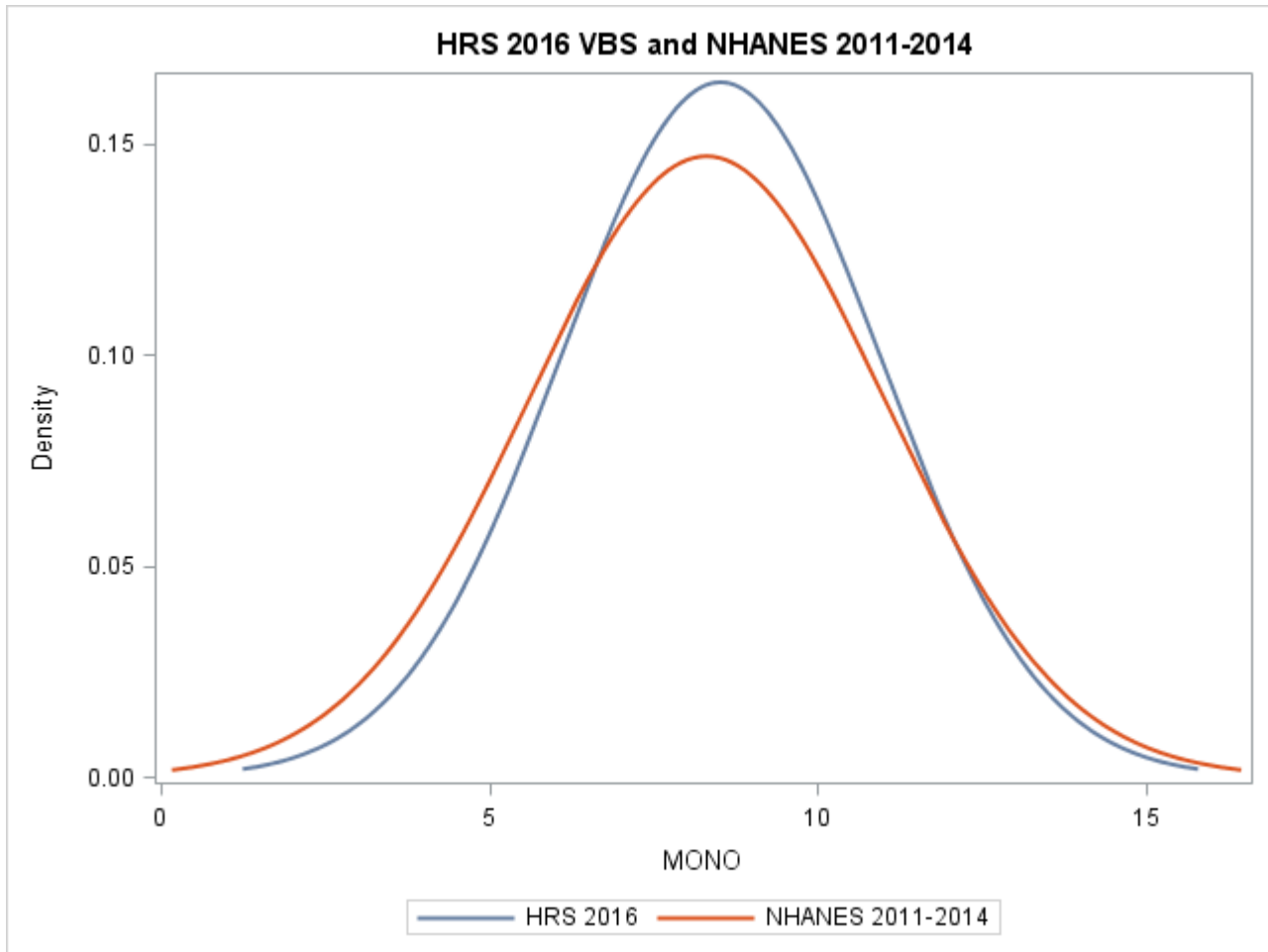




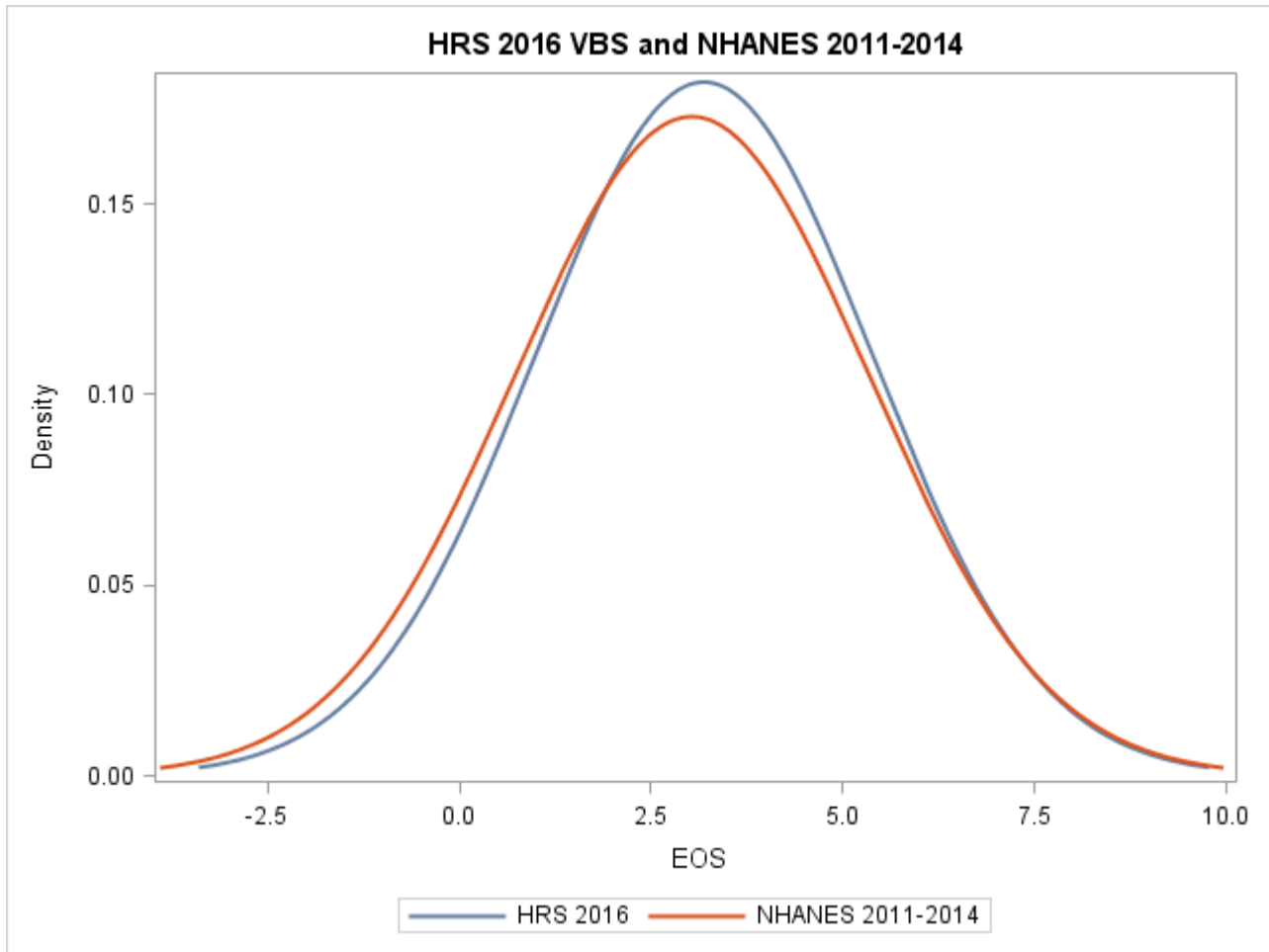
# % Lymphocytes



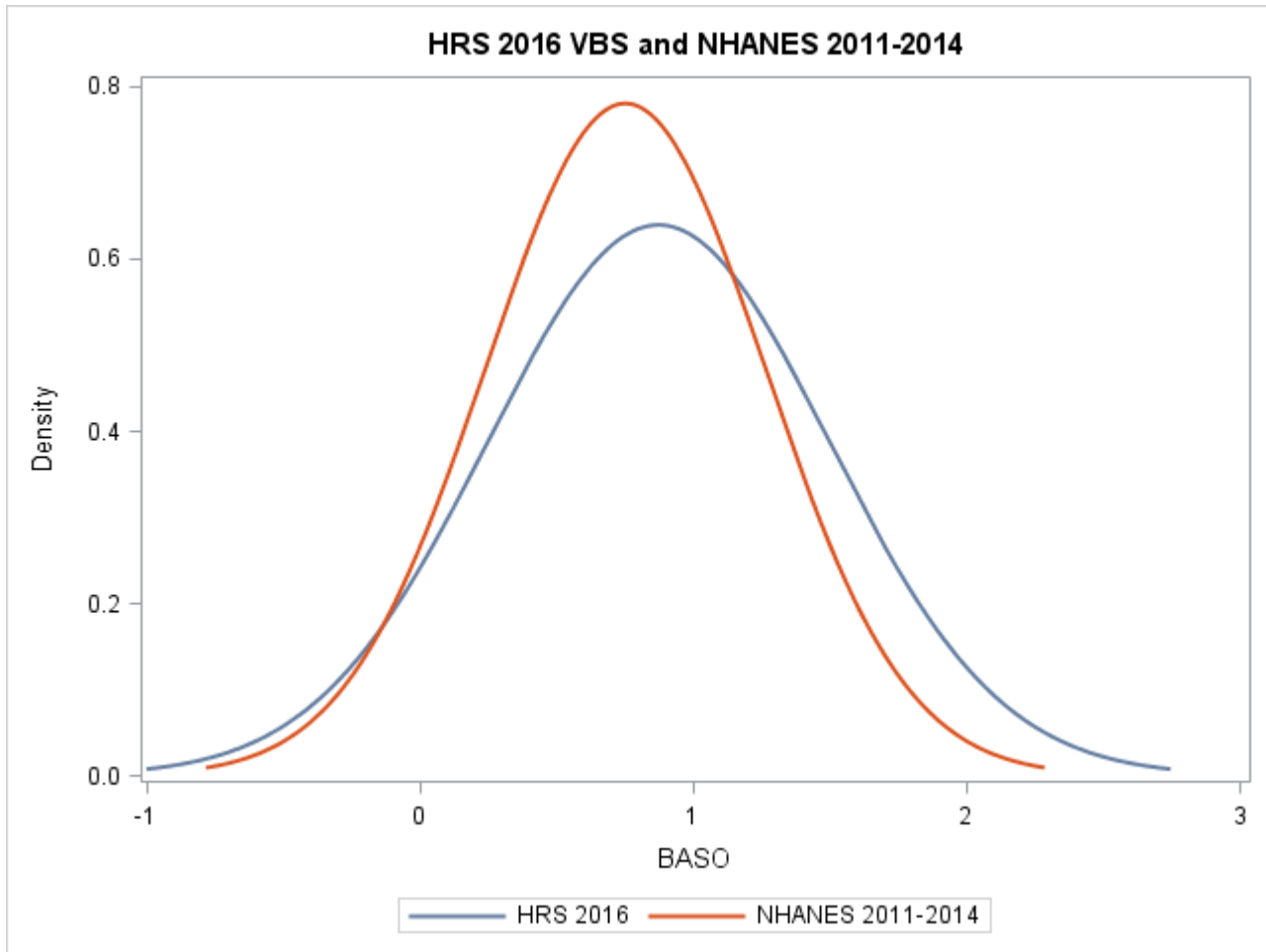
**% Monocytes**



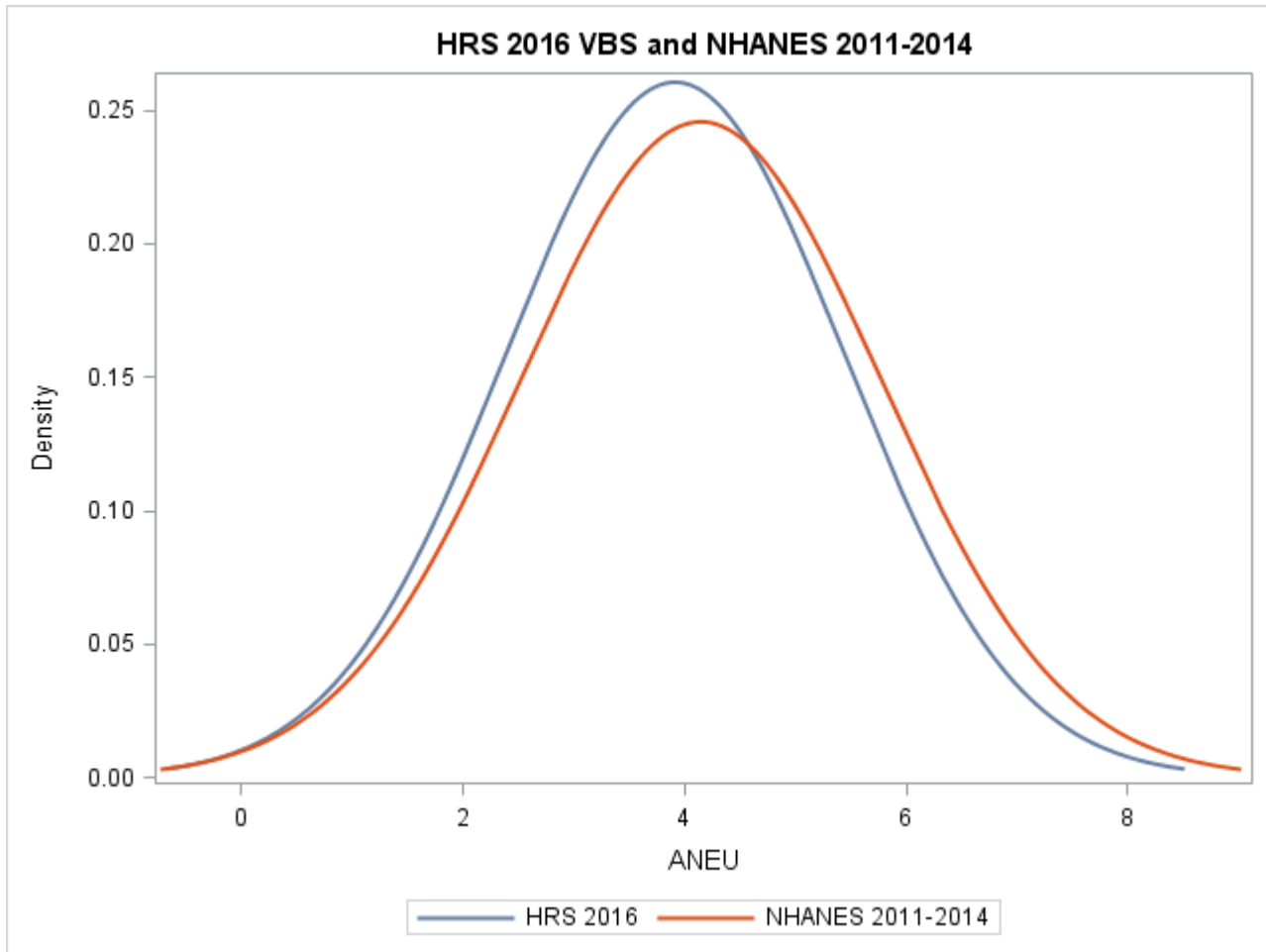
**% Eosinophils**



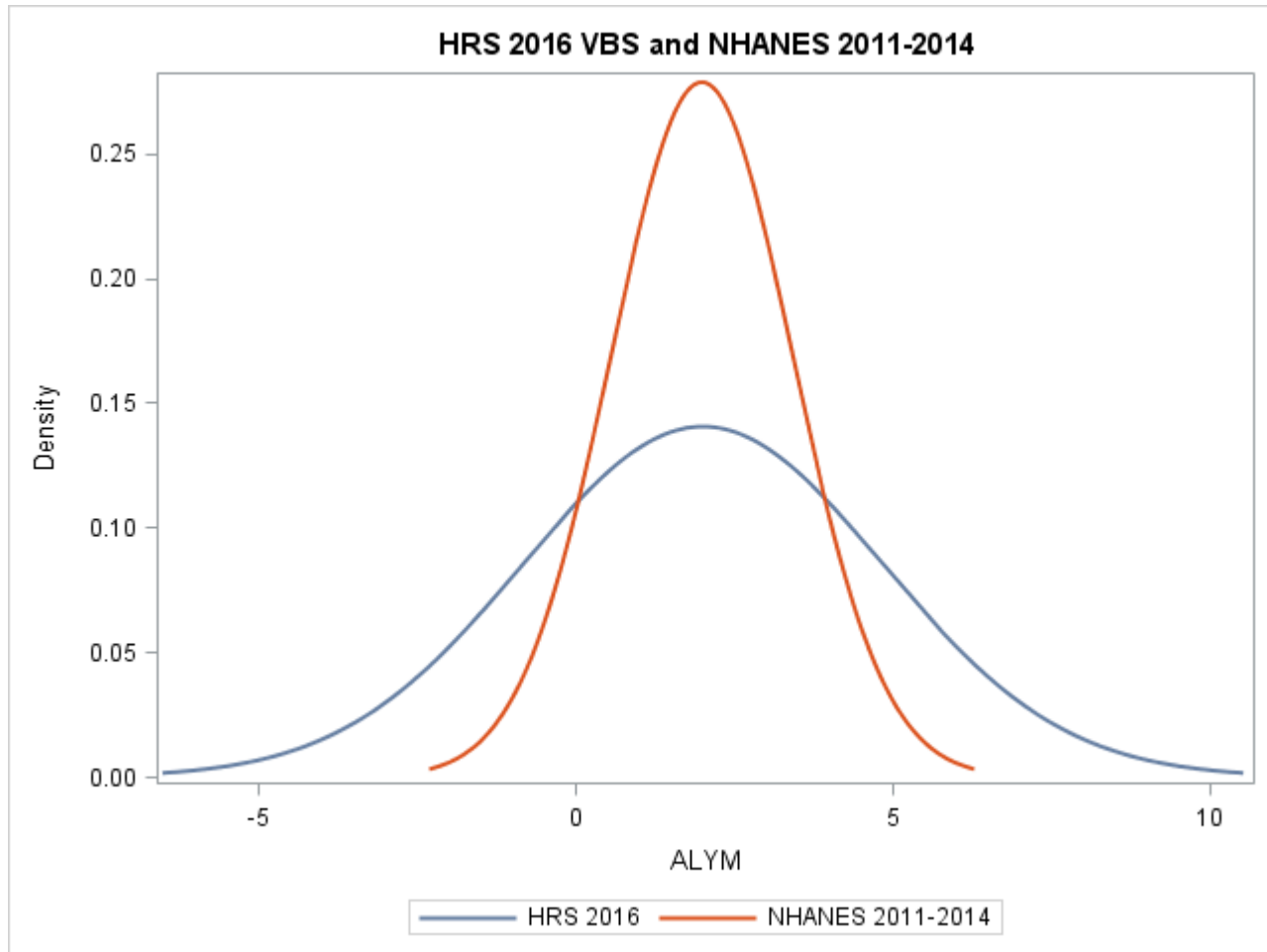
# % Basophils



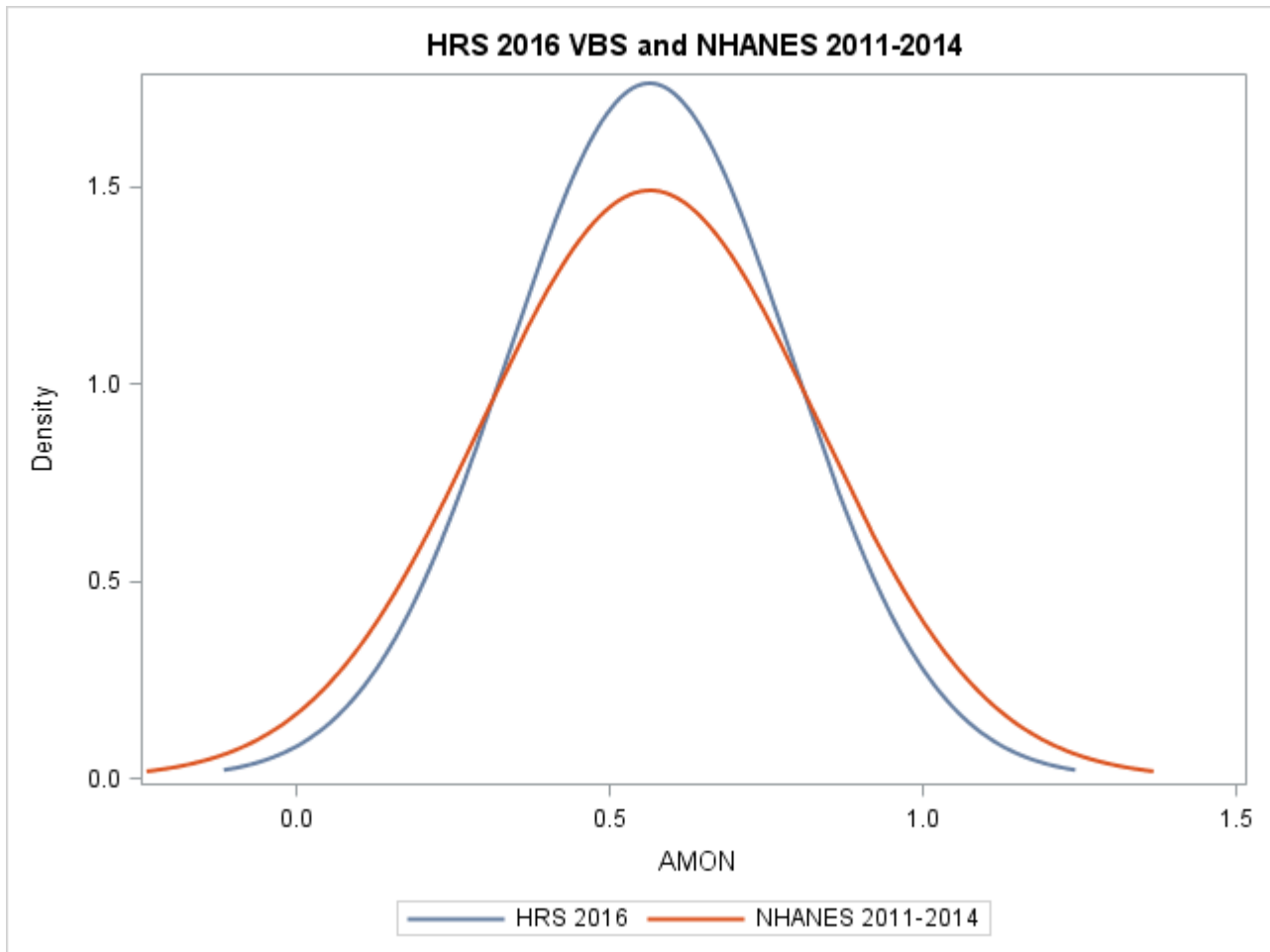
## Neutrophil Count



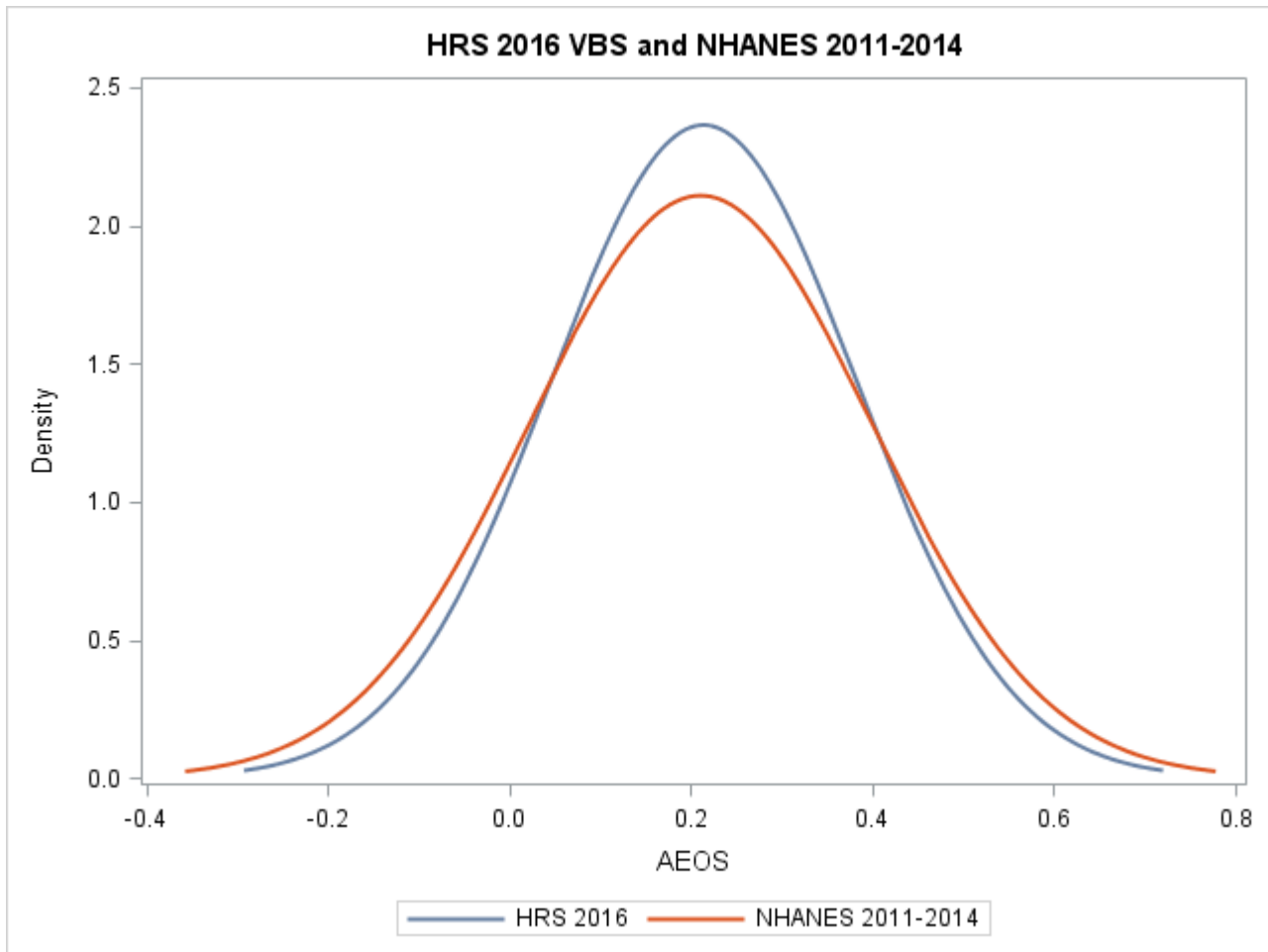
# Lymphocyte Count



## Monocyte Count

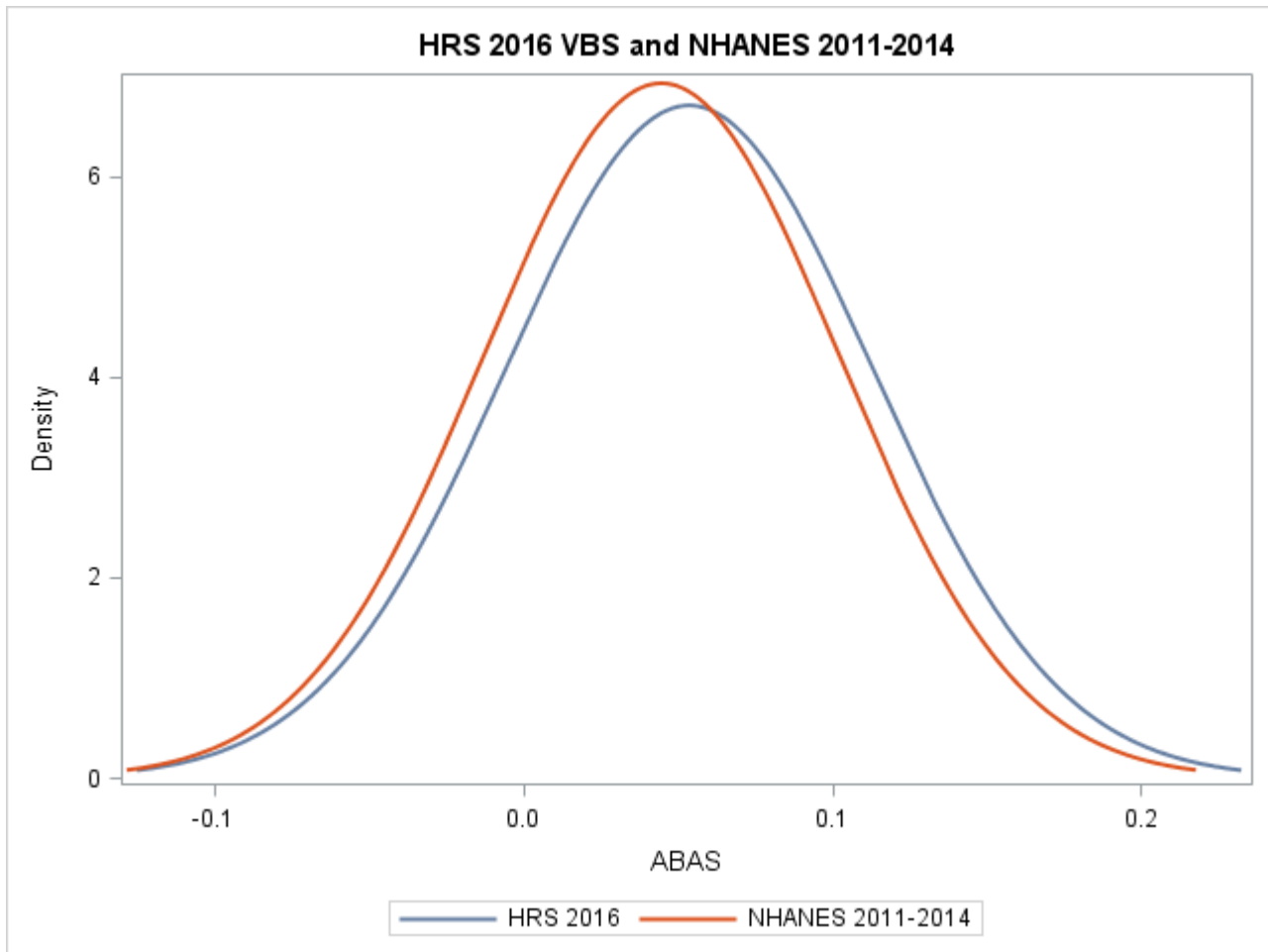


## Eosinophil Count

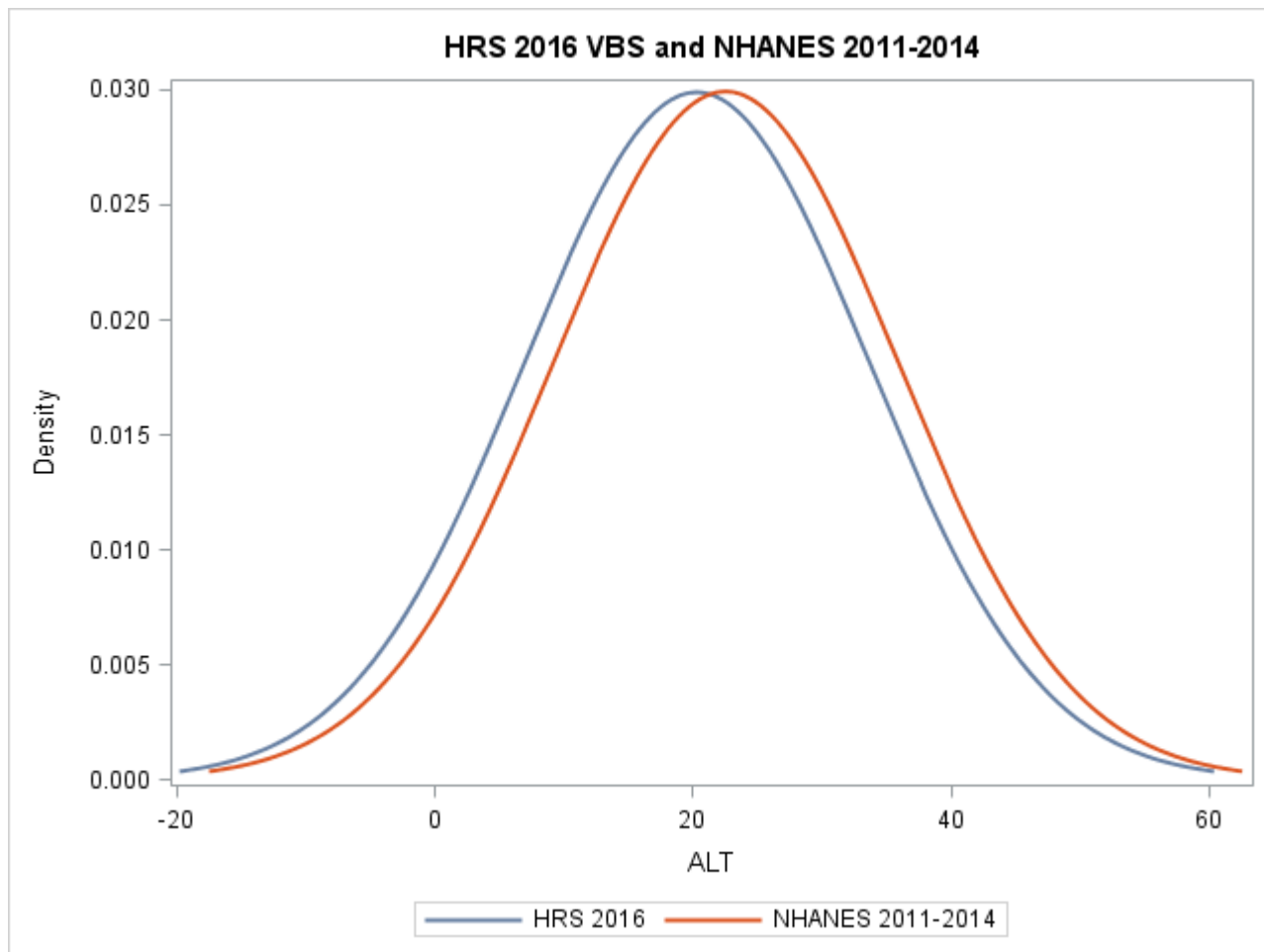




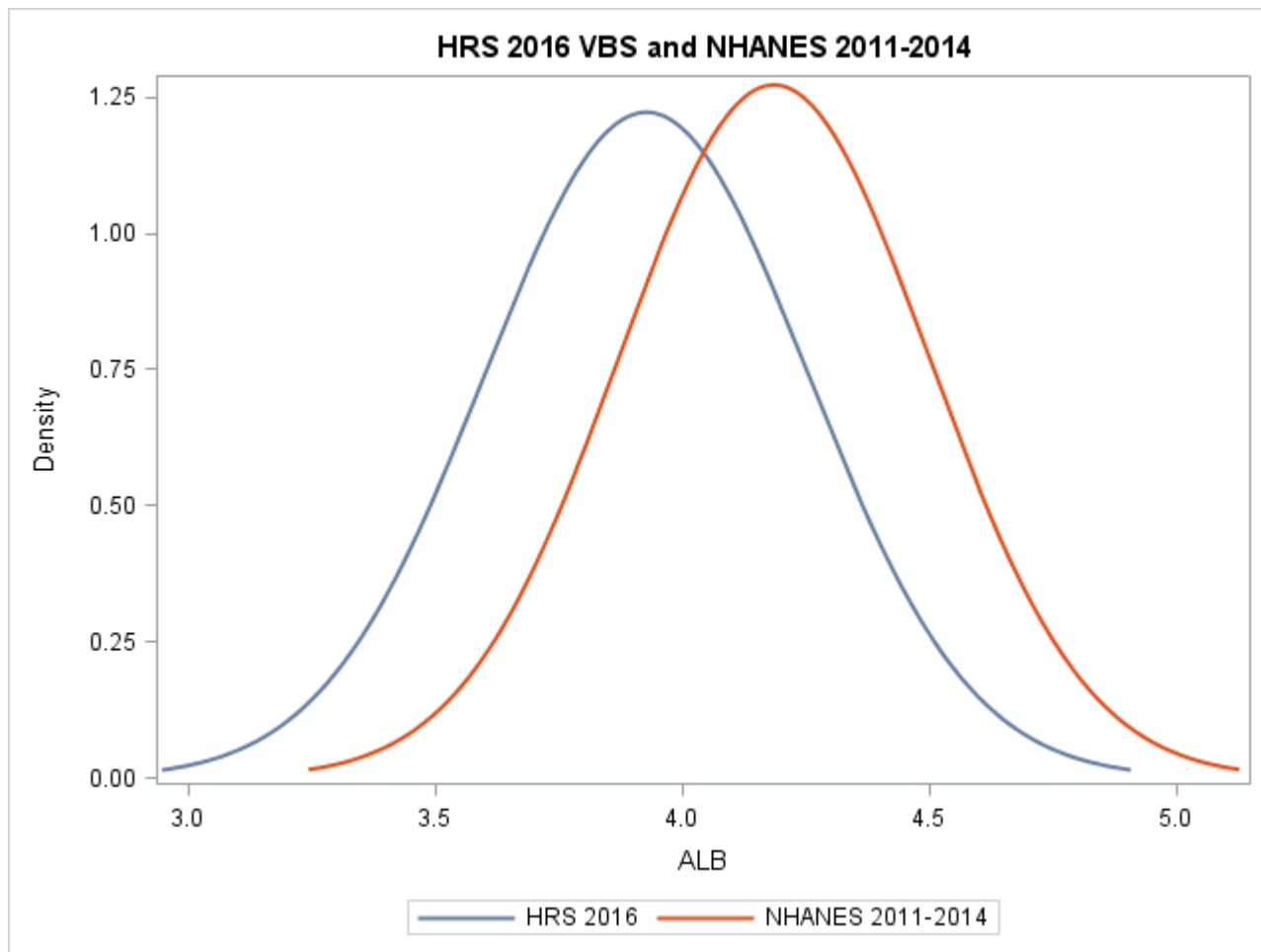
## Basophil Count



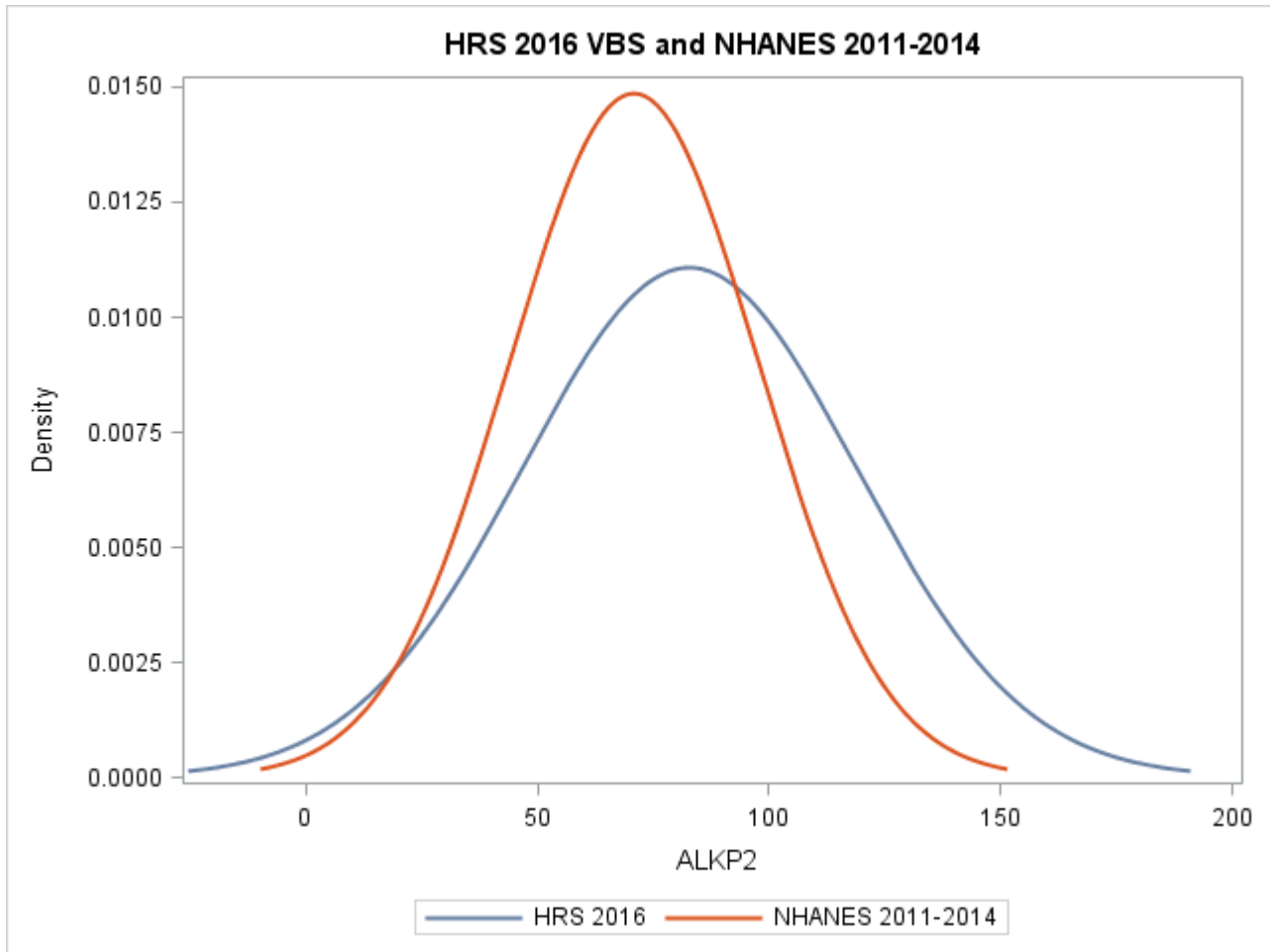
# Alanine Aminotransferase



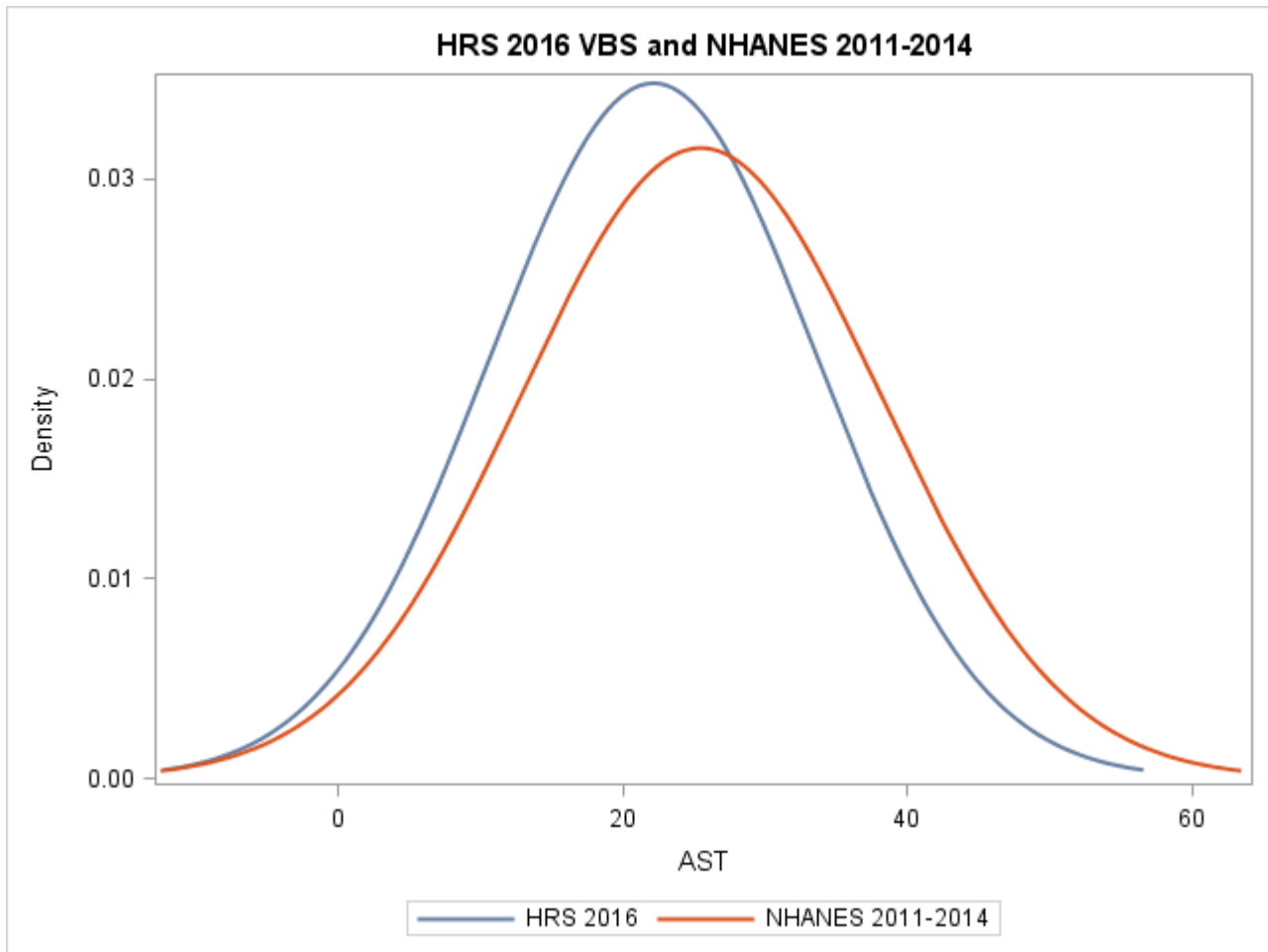
## Albumin



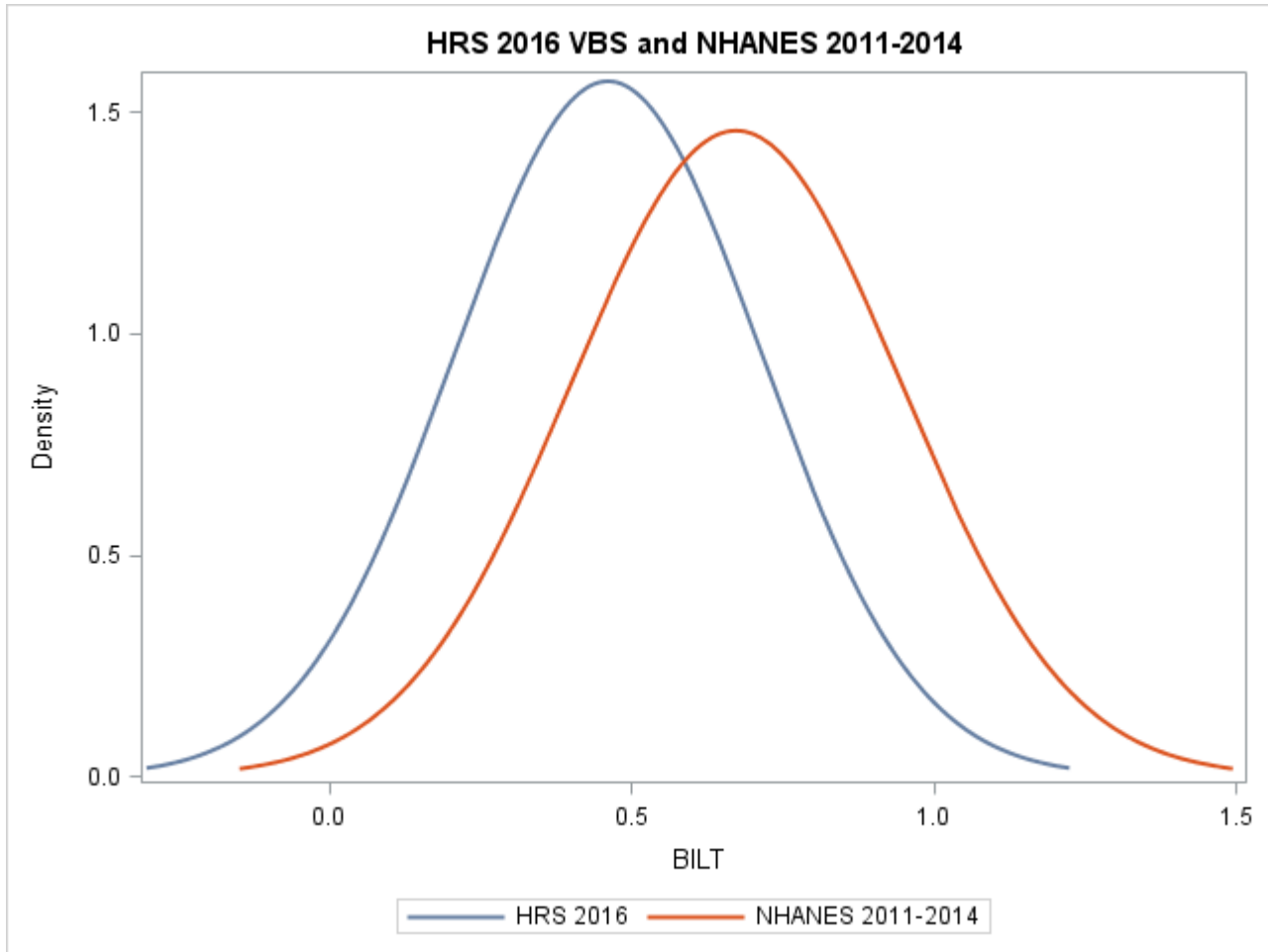
## Alkaline phosphatase



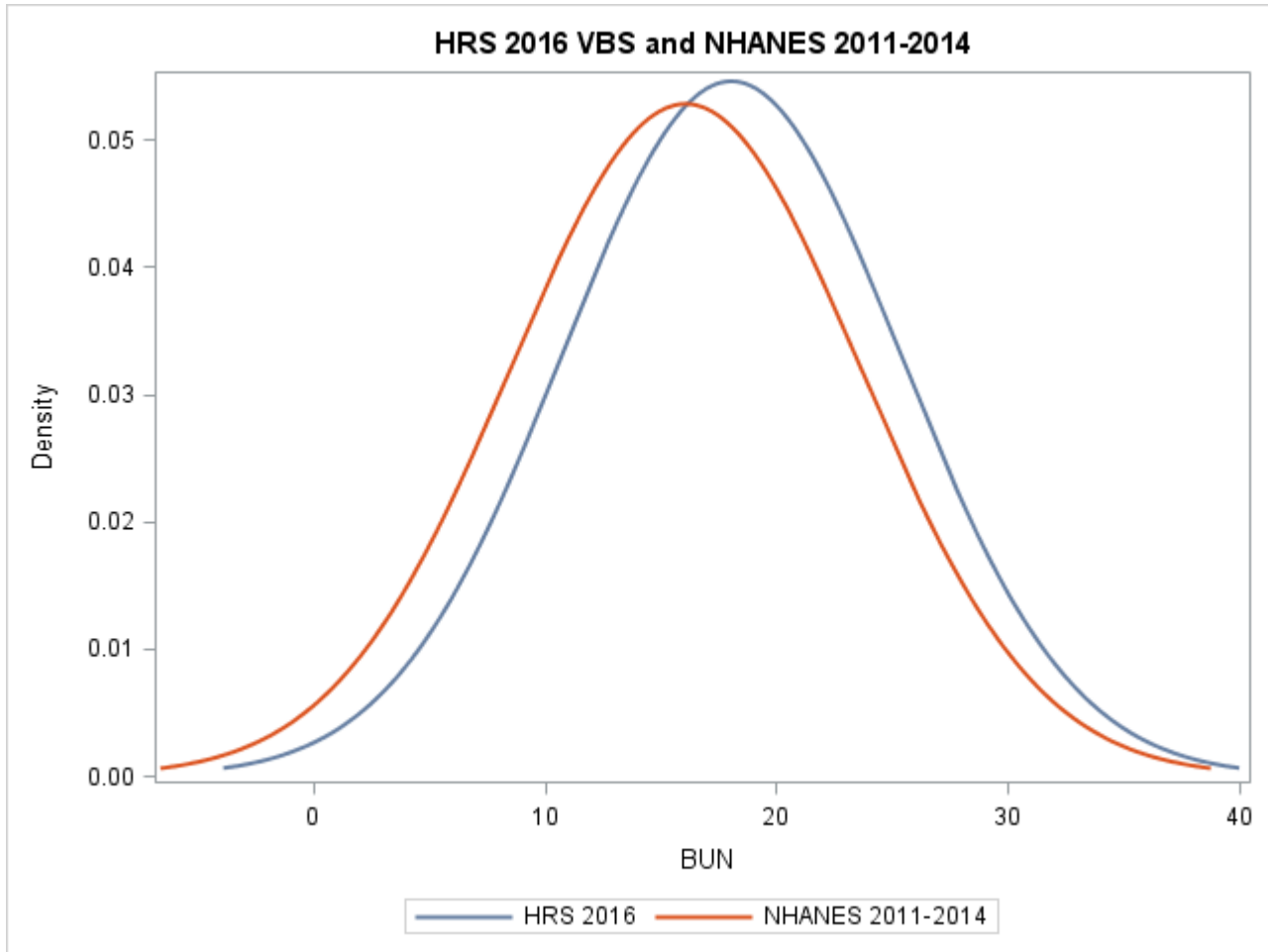
# Aspartate Aminotransferase



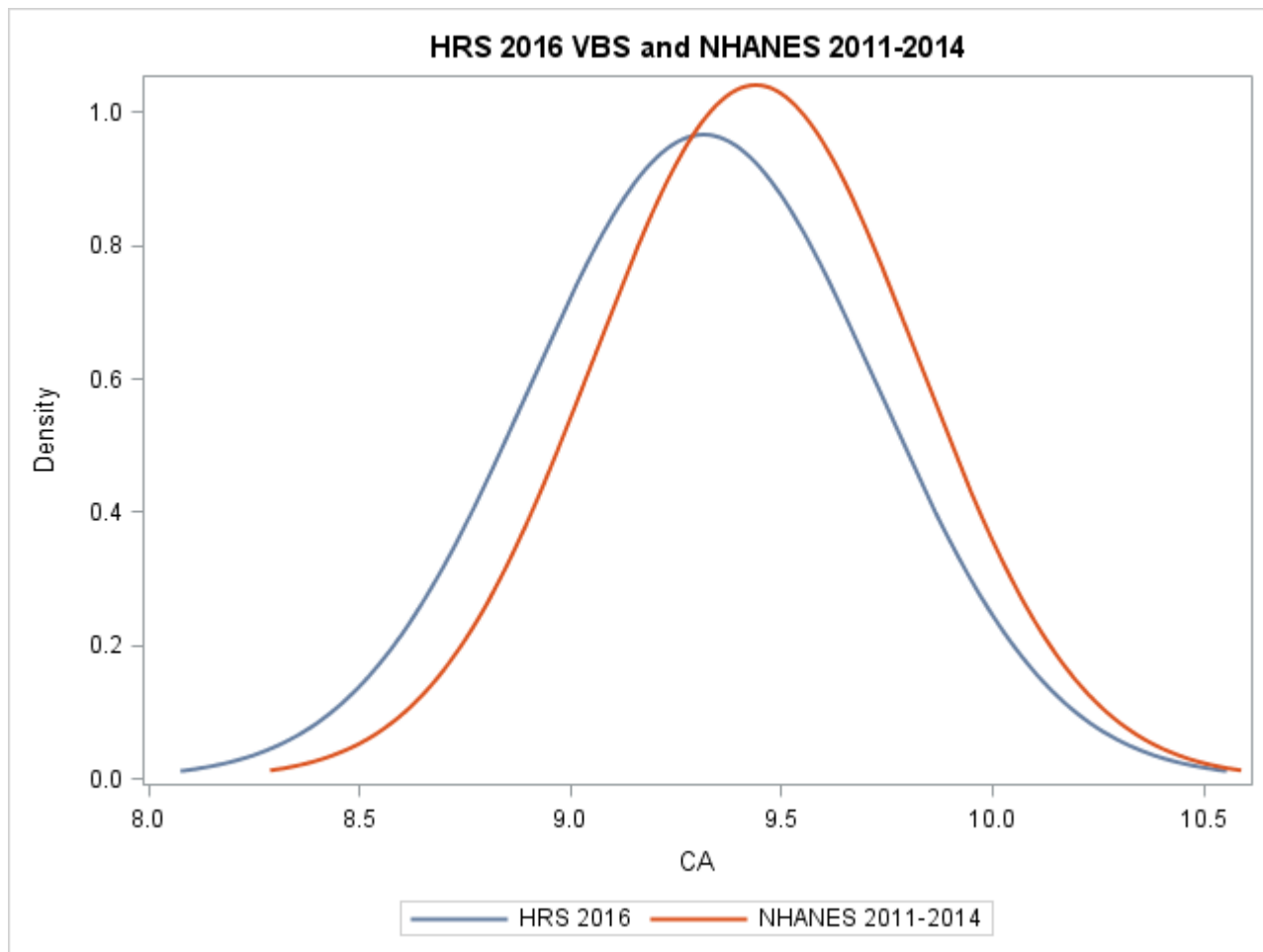
**Bilirubin,Total**



# Urea Nitrogen (BUN)

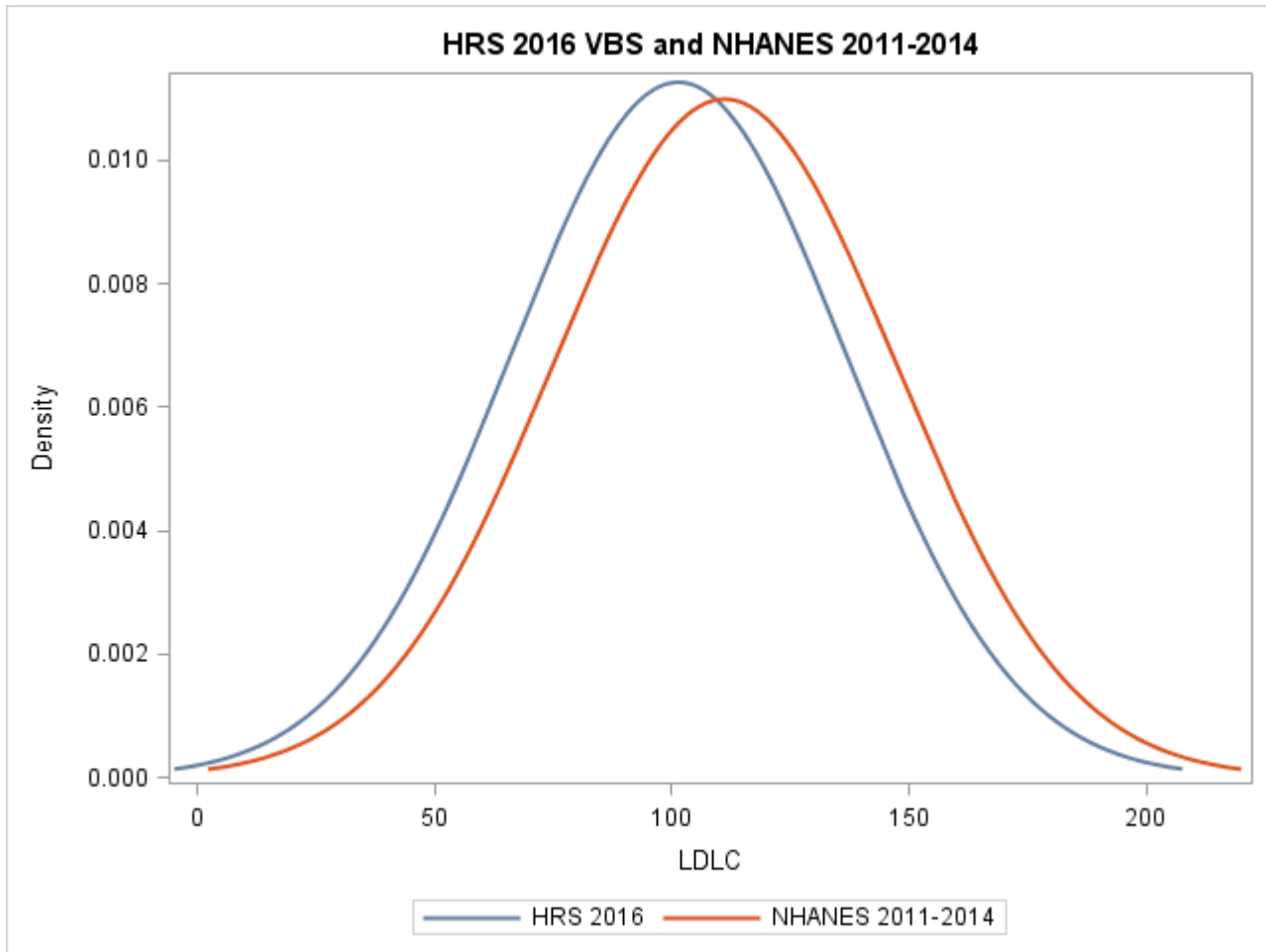


# Calcium

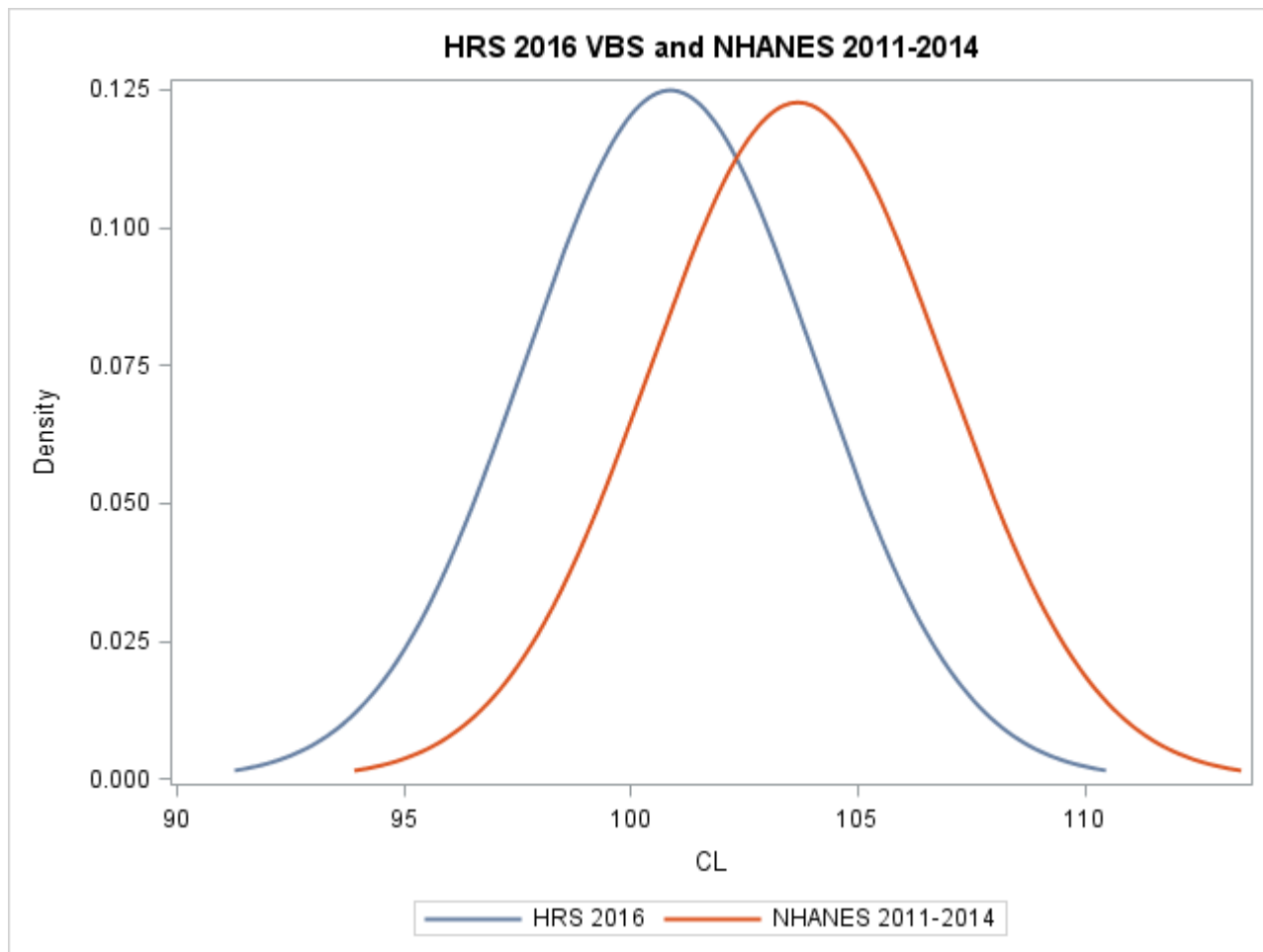




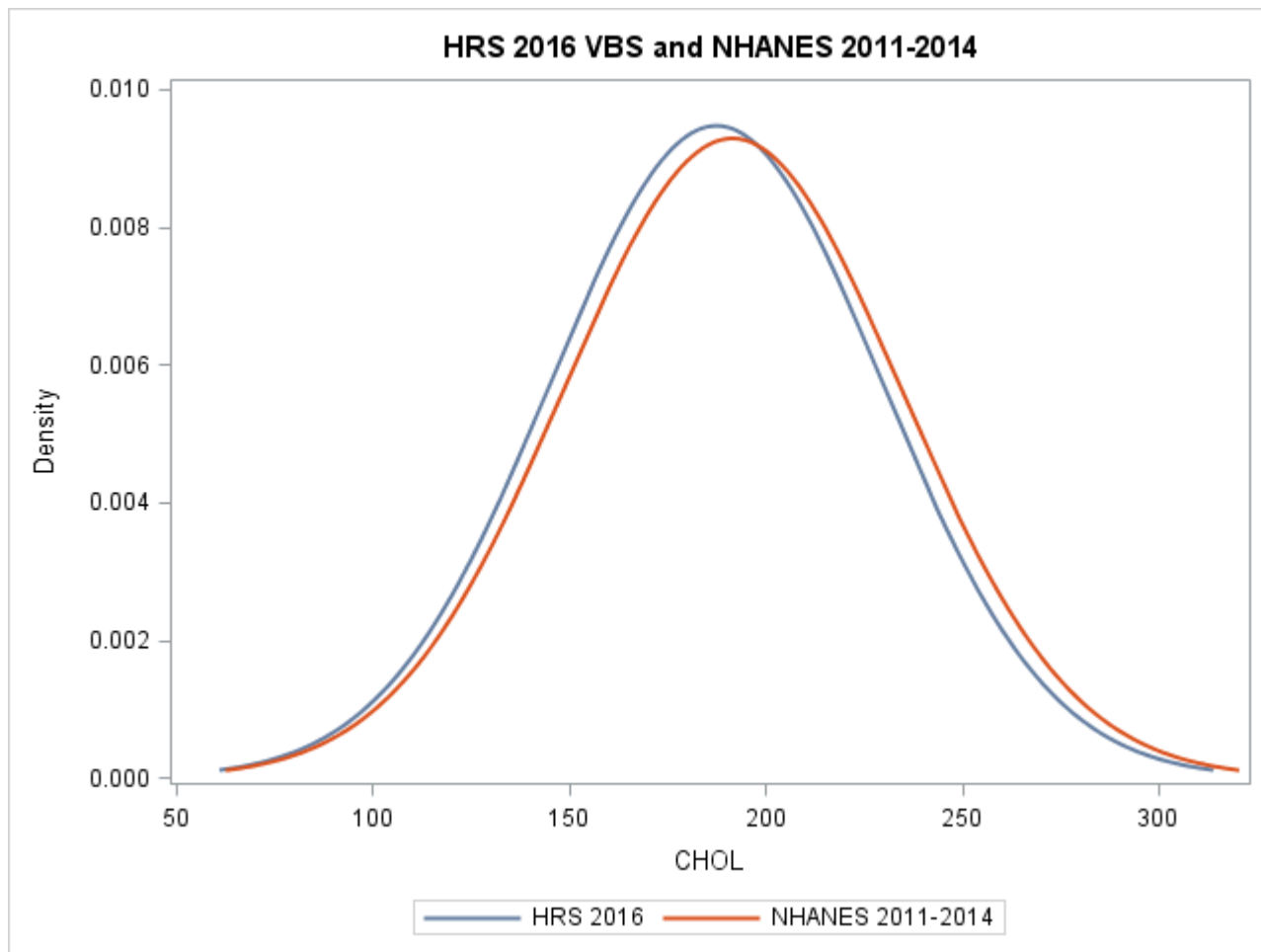
## LDL-Cholesterol



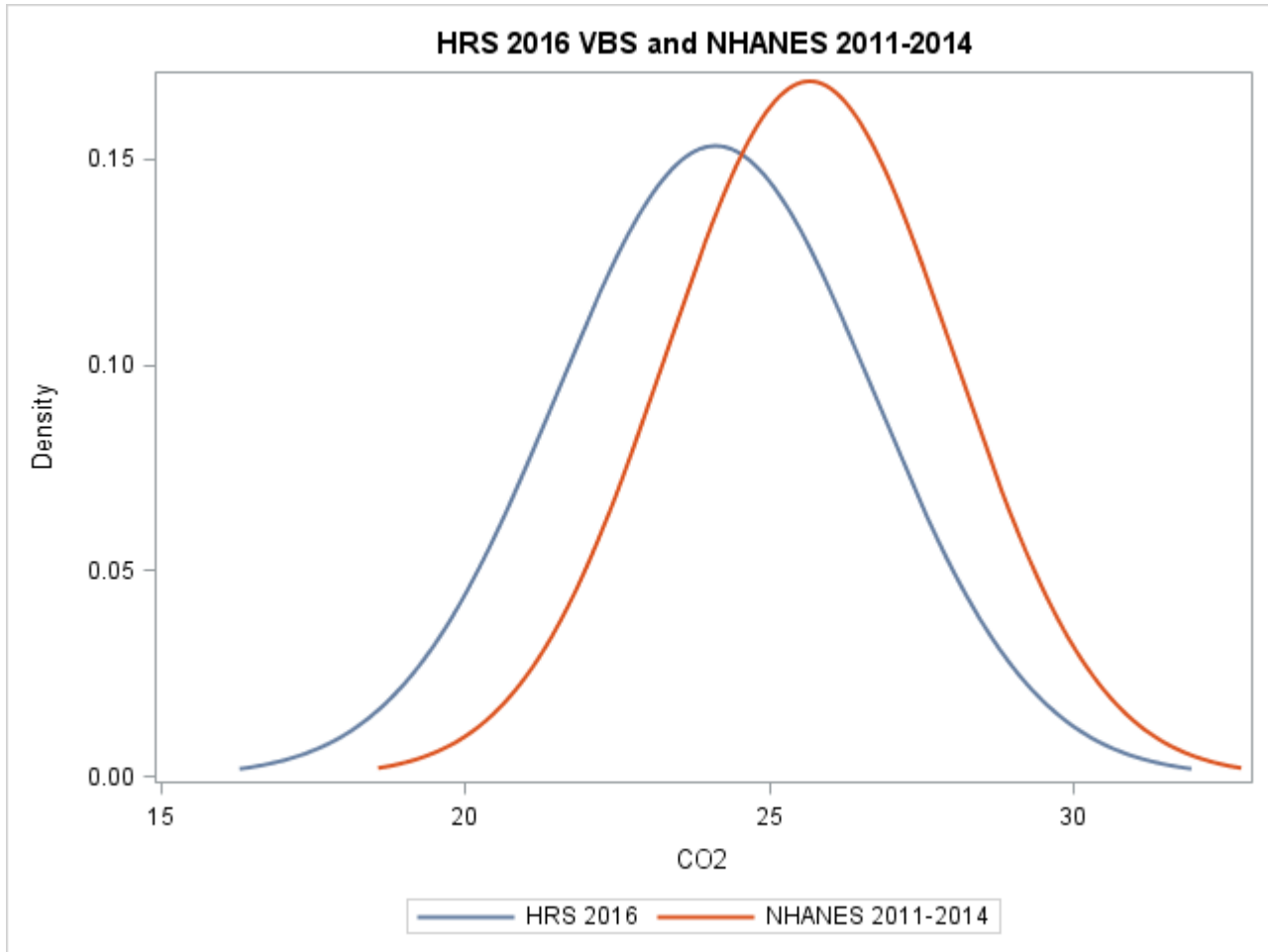
# Chloride



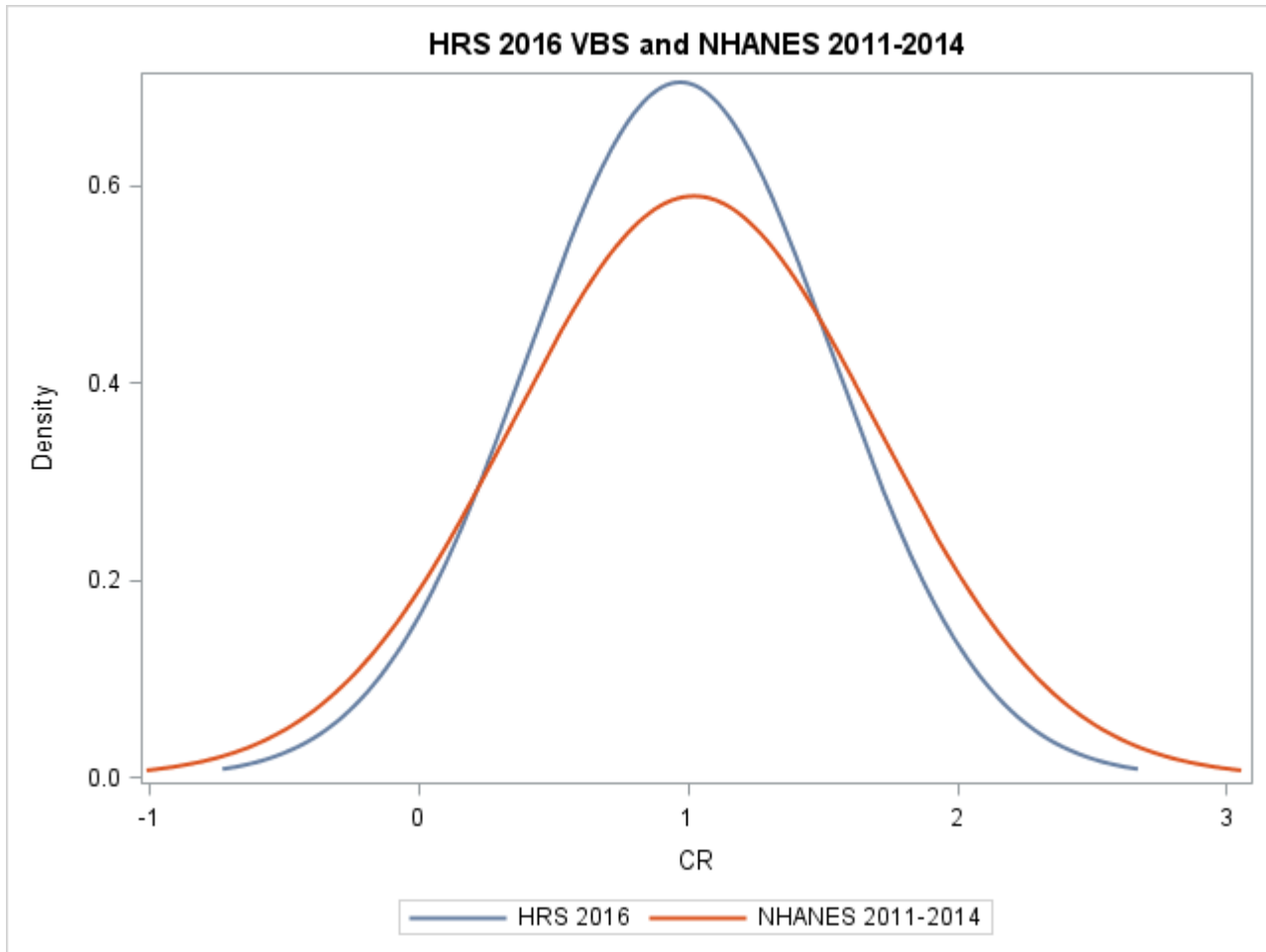
## Total Cholesterol



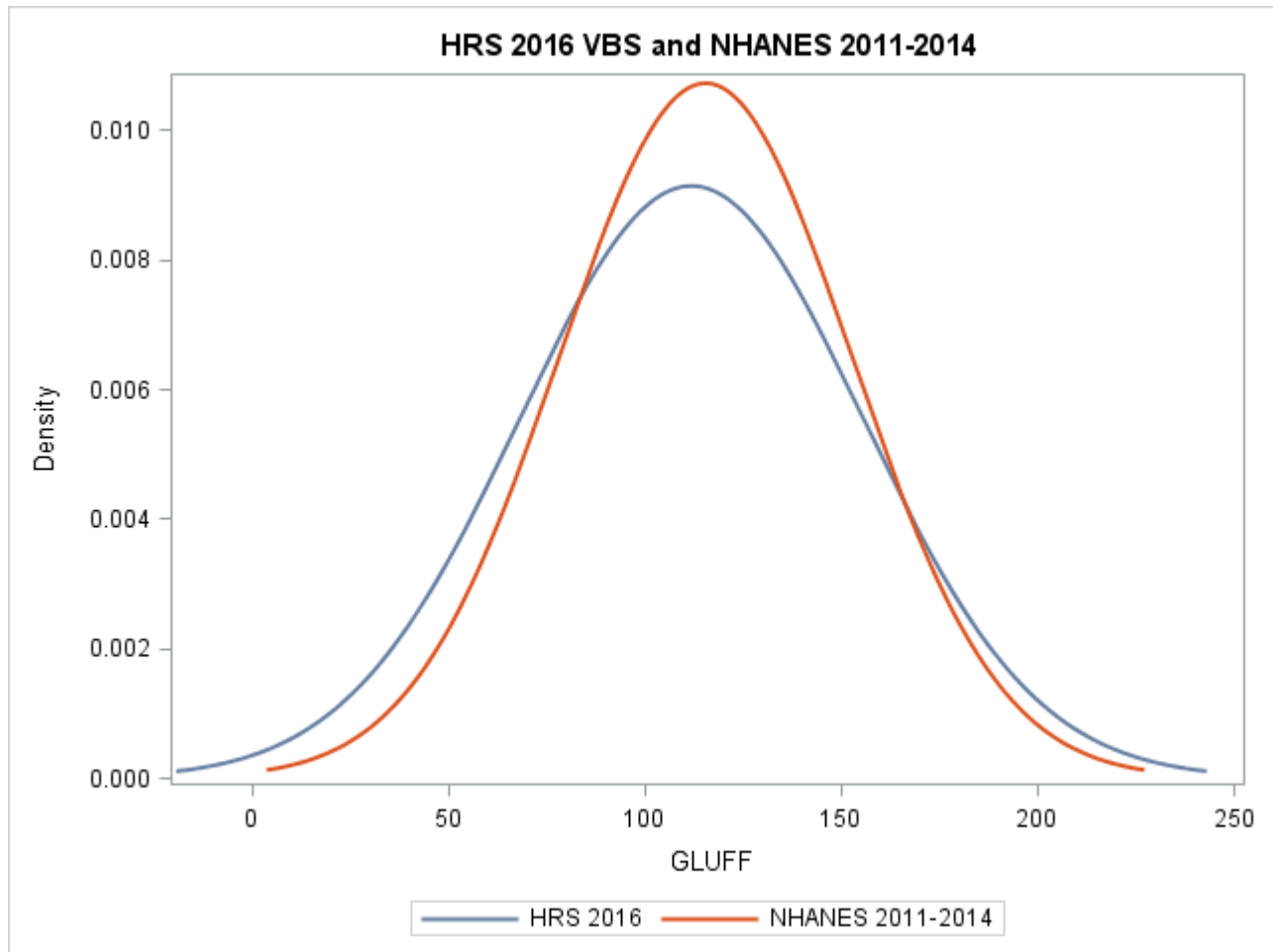
**Bicarbonate (CO2)**



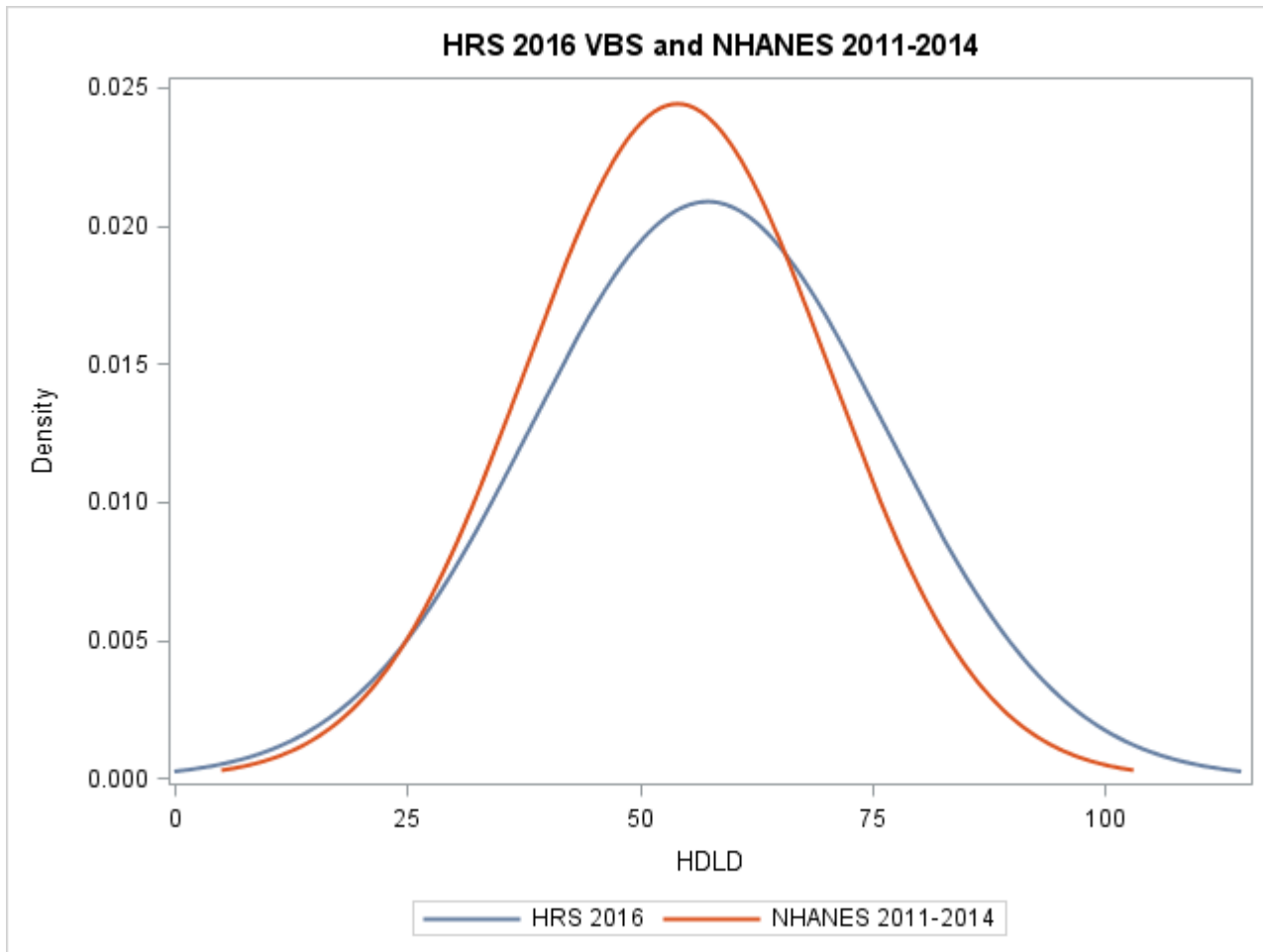
## Creatinine



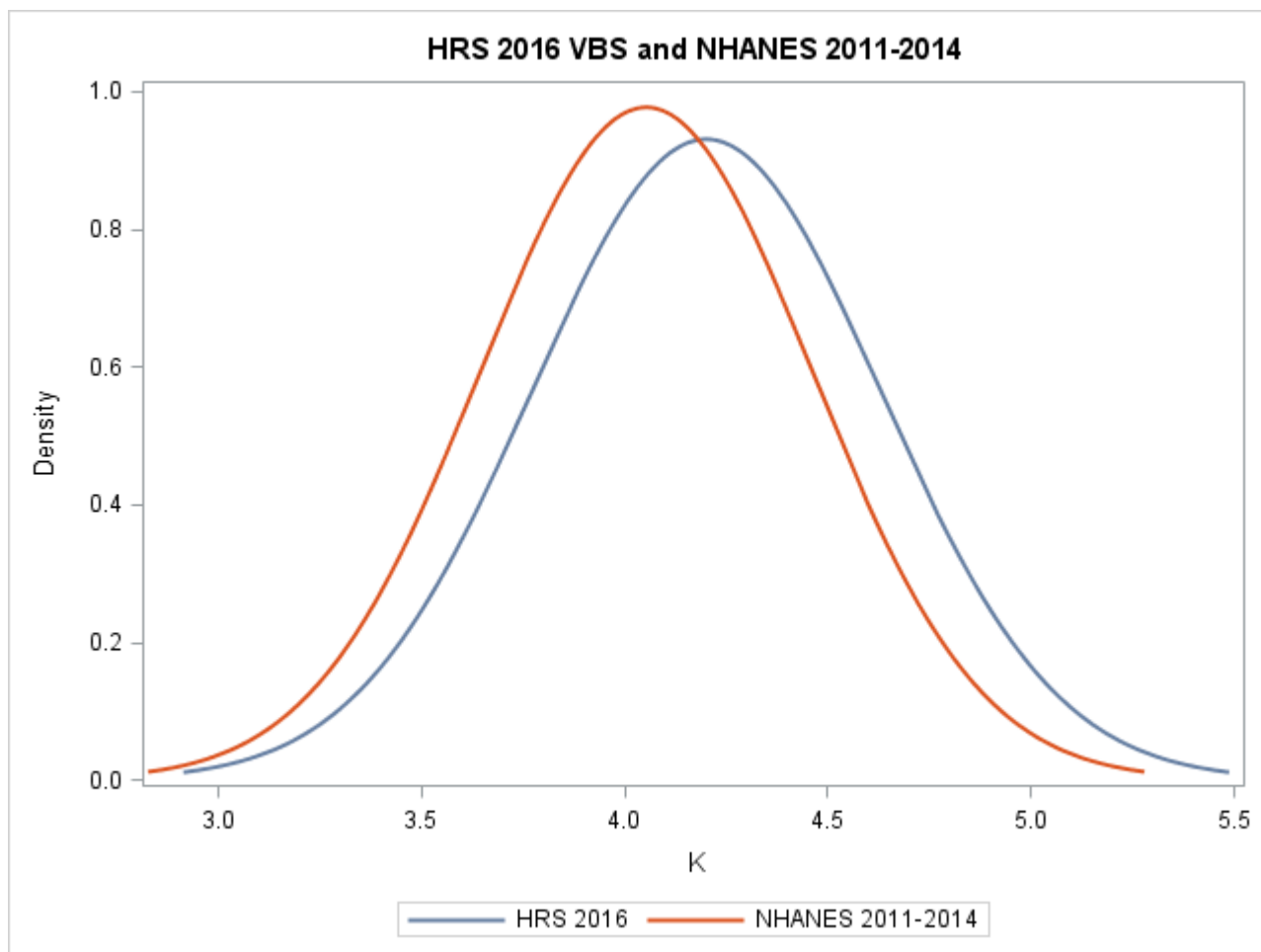
## Glucose, Fasting



# HDL-Cholesterol

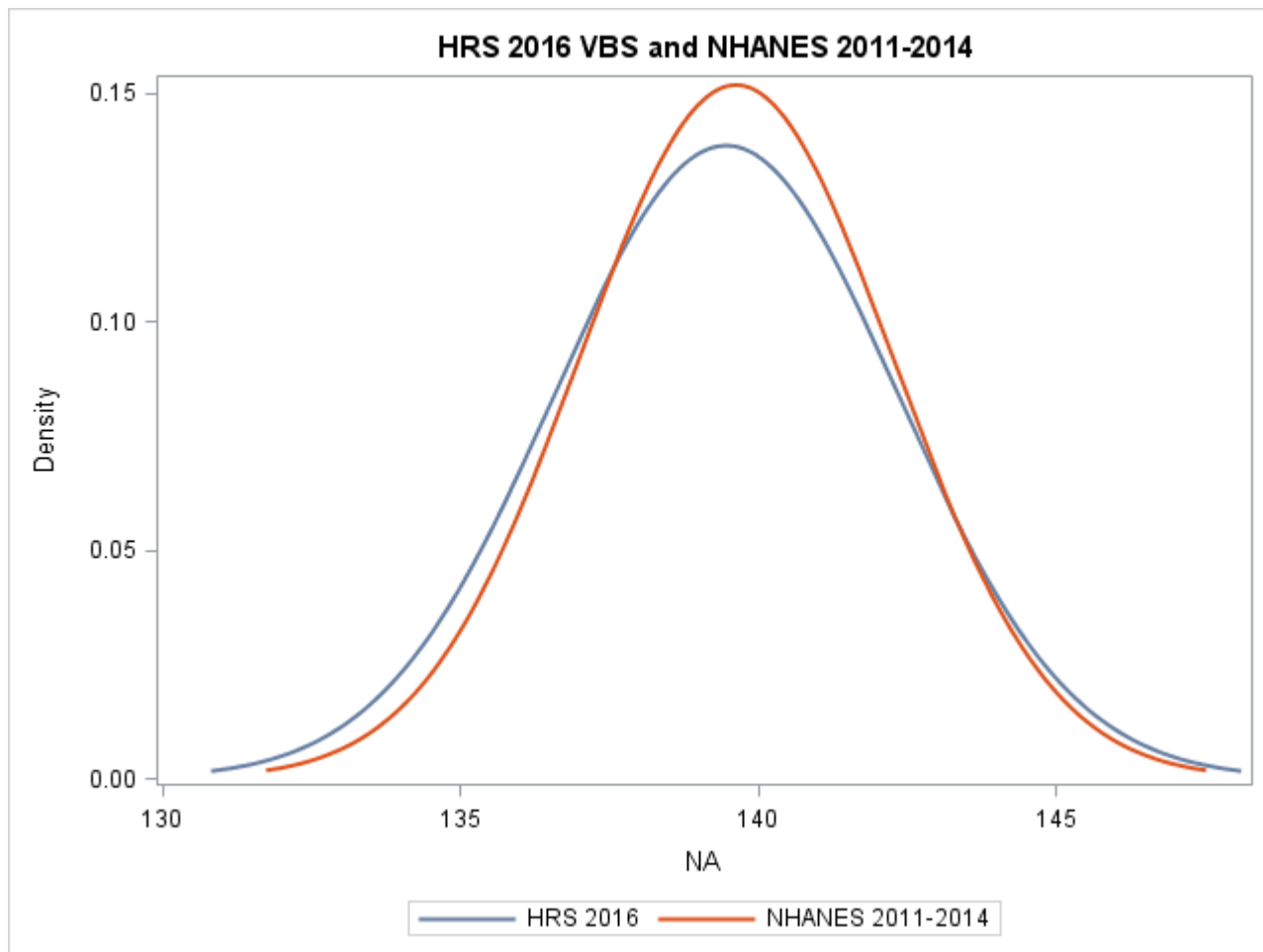


# Potassium

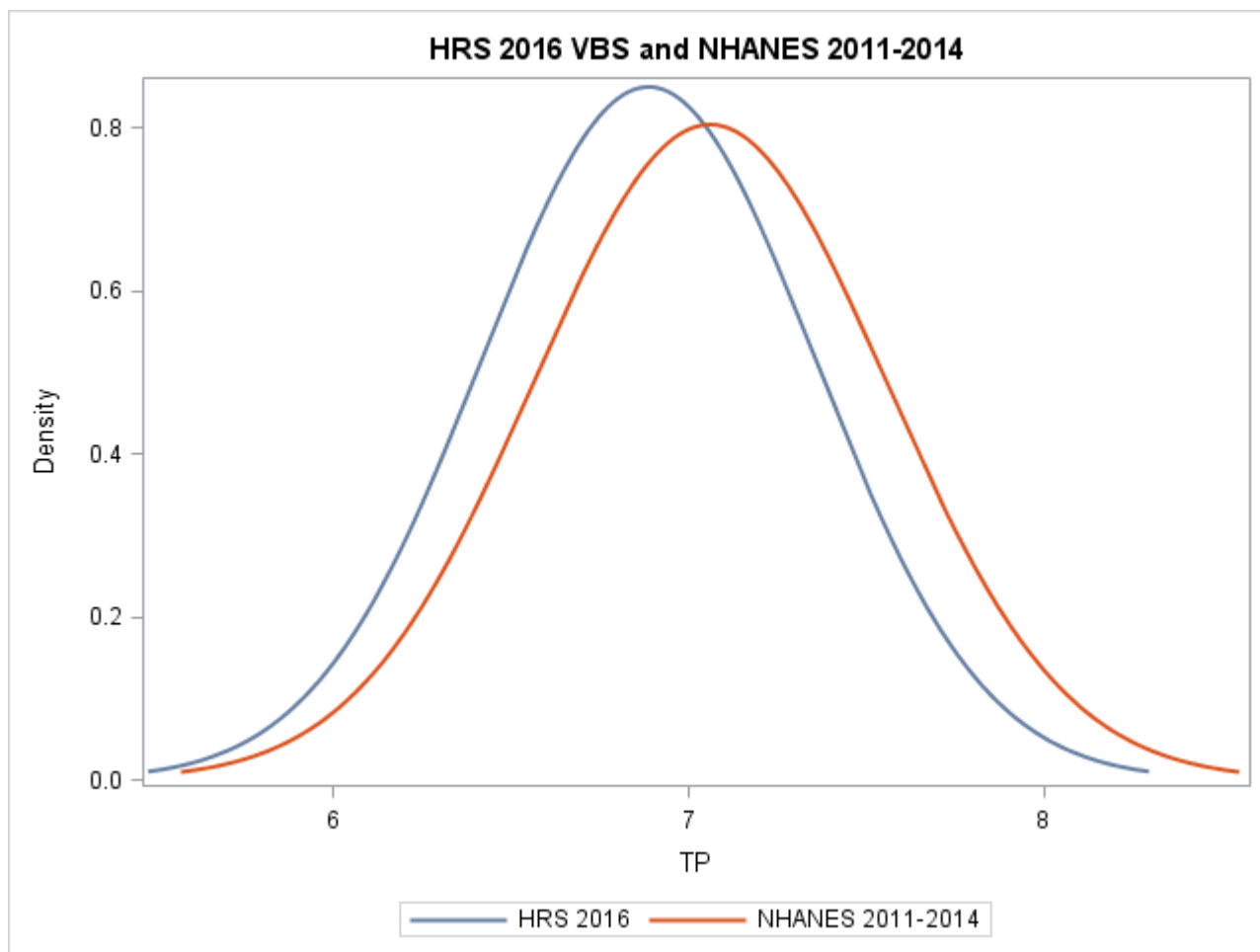




## Sodium



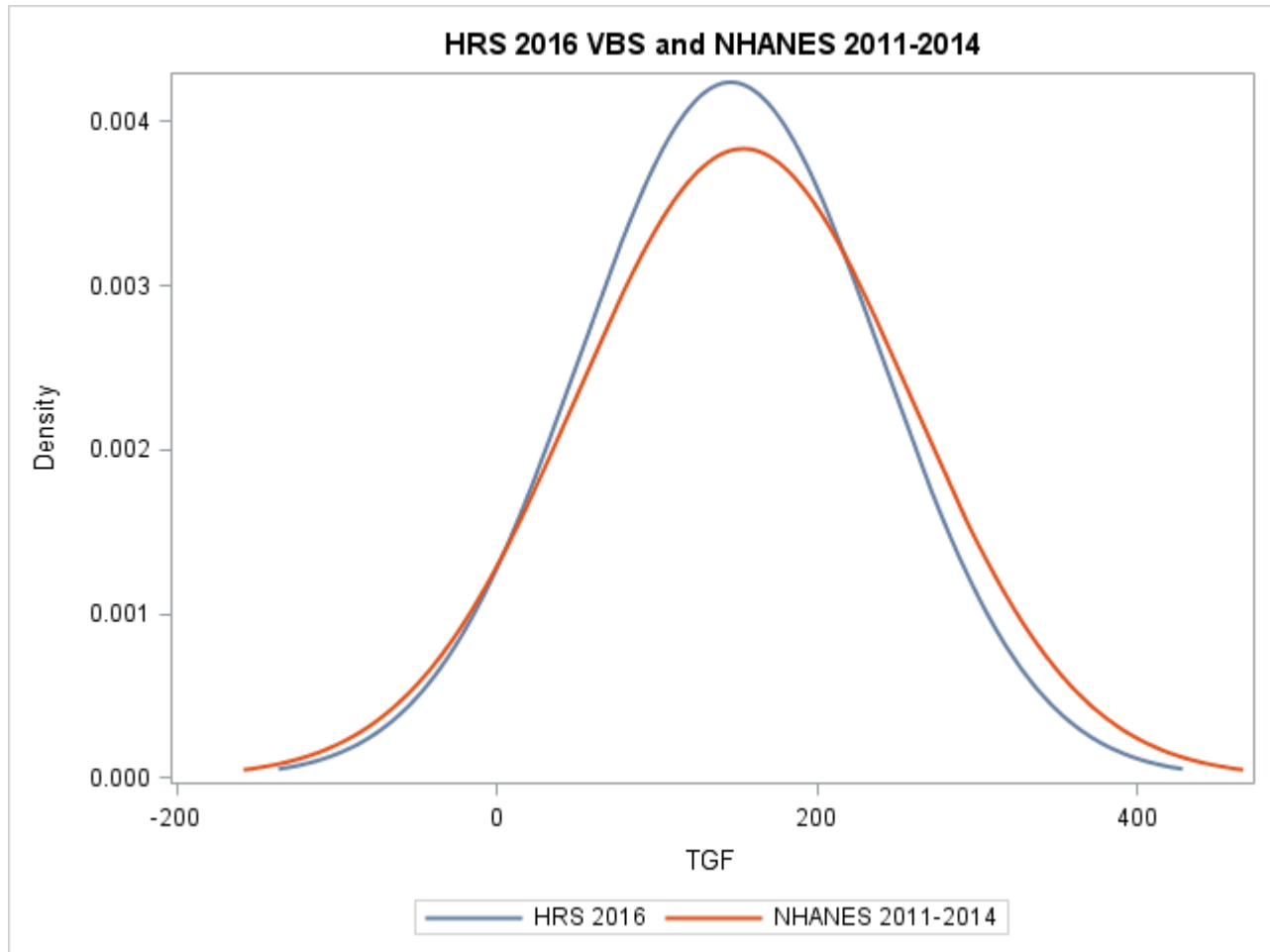
## Total Protein



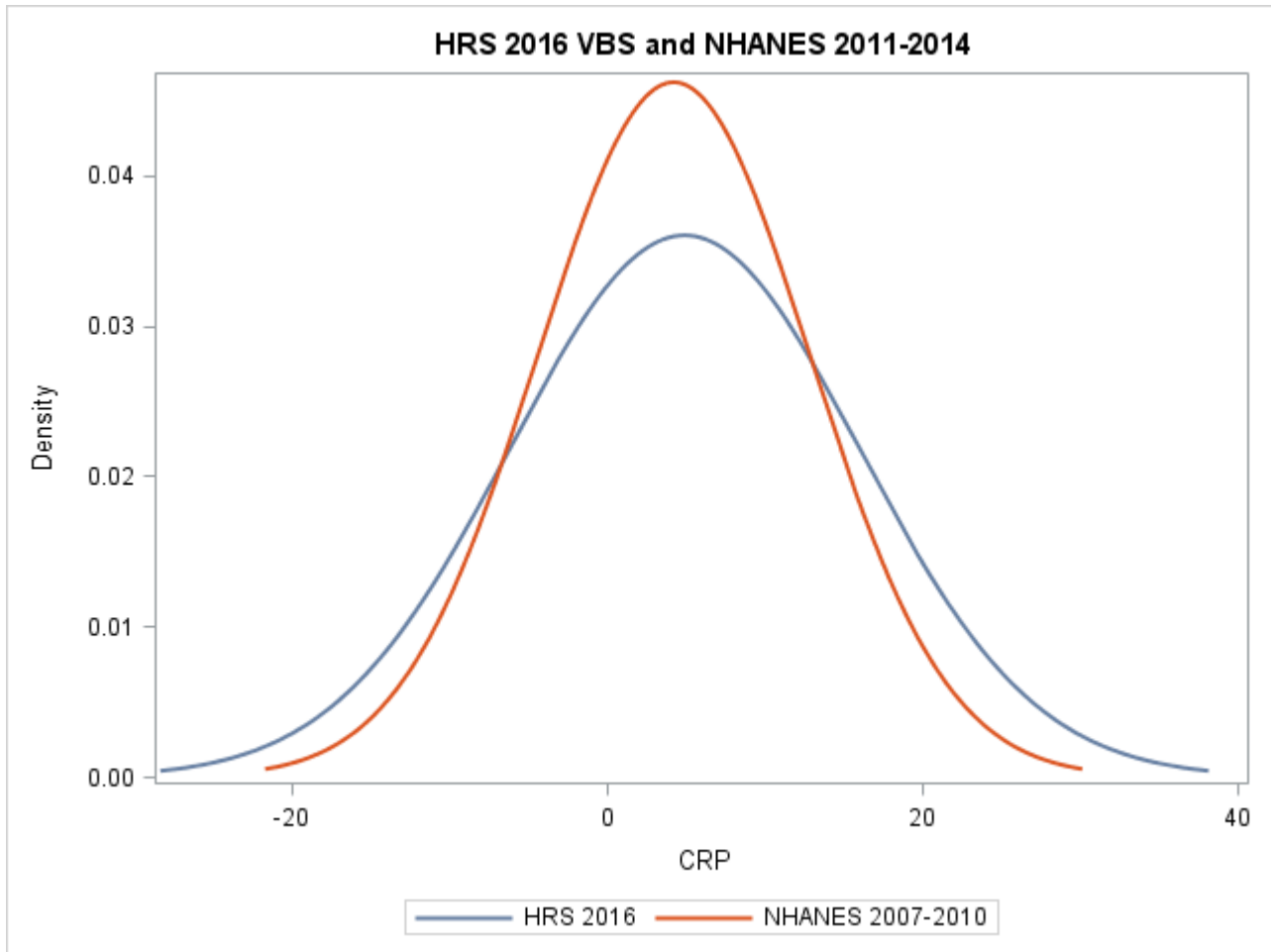
HRS's unit is g/dL; NHANES unit is g/L.

NHANES unit changed from g/L to g/dL

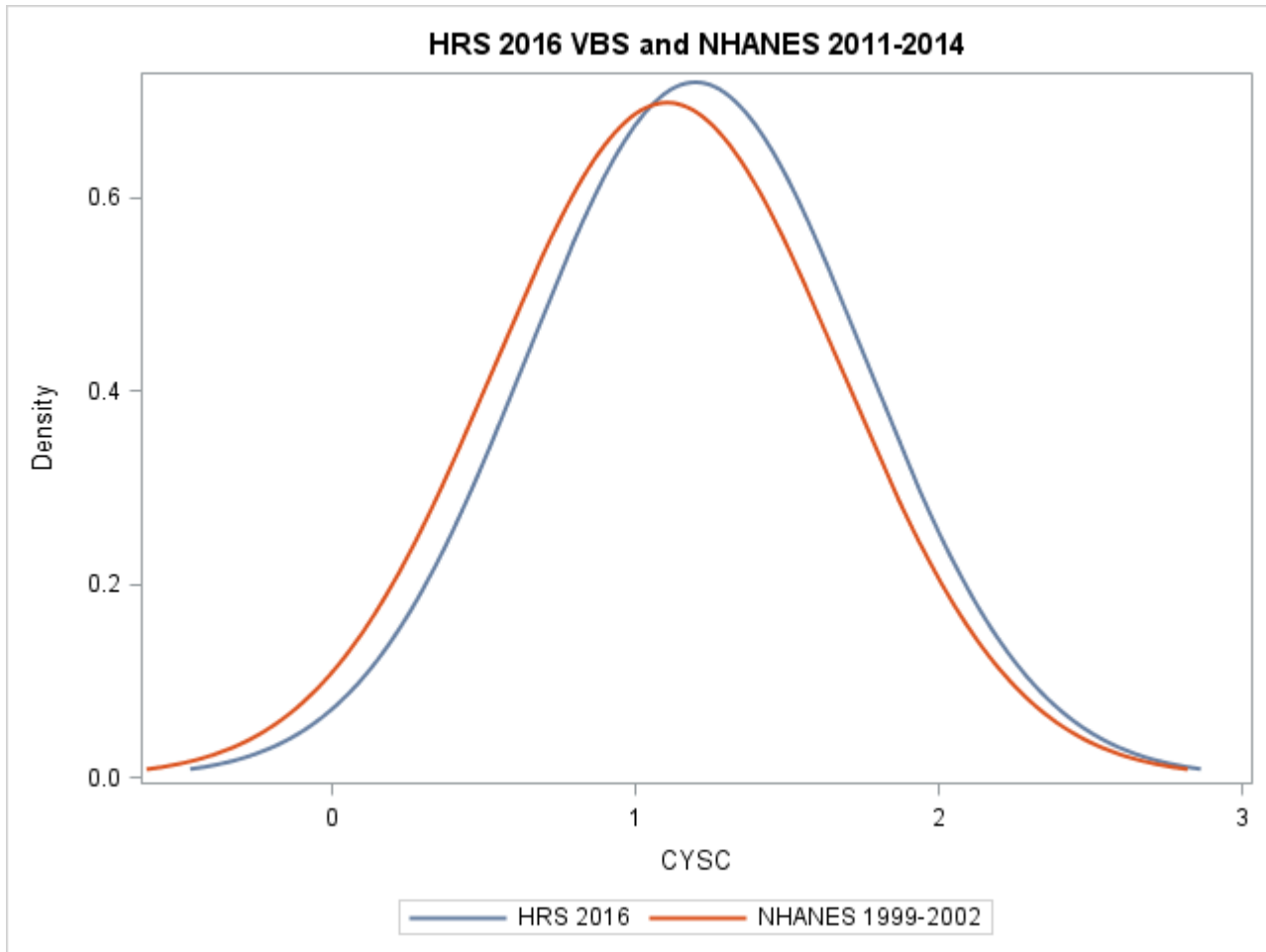
# Triglycerides



# CRP



## Cystatin C



## Early Descriptive Results Selected Flow Cytometry Markers N=940.

We provide a table of descriptive results for a selected subset of measures of immune function from the first 1,000 cases. We also find significant links to age, sex, race and education for these measures.

<b>Variable</b>	<b>N</b>	<b>Mean</b>	<b>Std Dev</b>	<b>Minimum</b>	<b>Maximum</b>
<b>Central Memory cytotoxic T cells</b>	940	0.0372059	0.0487087	0.000067000	0.5105914
<b>Effector (E) Cytotoxic T cells</b>	940	0.1670595	0.2006430	0.000827000	1.4489719
<b>Effector Memory (EM) Cytotoxic T cells</b>	940	0.0747065	0.0724200	0.000113000	0.5933446
<b>Central Memory Helper T cells</b>	940	0.3250452	0.2038249	0.0069678	2.1299154
<b>Effector Helper T cells</b>	940	0.0217846	0.0540262	0	0.7991029
<b>Effector Memory Helper T cells</b>	940	0.0580186	0.0881223	0.000399000	1.4319302
<b>Naïve Helper T cells</b>	940	0.3413953	0.2747497	0.0025902	2.5499818
<b>Natural Killer lymphocytes</b>	940	0.2491240	0.1644850	0.0023000	1.9033000
<b>CD56HI Natural Killer lymphocytes</b>	940	0.0066423	0.0078322	0	0.1207000
<b>CD56LO Natural Killer lymphocytes</b>	940	0.2226770	0.1533360	0.000800000	1.8329000

## **Repository Management**

The University of Minnesota acts as a repository for VBS blood. Respondents who have consented to future use of their samples within the broad aims of the HRS to study health and aging have serum, plasma, RNA and DNA stored from this collection. Access to the repository samples is managed by HRS. That means future analysis will most likely come about from future competing revision or renewal applications made by HRS for funding. Separately funded projects would be considered only if they were entirely in keeping with the aims of HRS, including public release of data. Whatever the origin of a project, all future use will require the full approval of the HRS PI and the NIA, which generally relies on review by the HRS Data Monitoring Committee.

### Future Proposed Blood Collection

During the next 6 year cycle we've propose to collect venous blood from each respondent one time. Of these approximately 7,300 will be repeats and 5,000 will be first-time. Our first priority will be widening our coverage. Blood samples will be collected in 2018 from the 2016 new cohort and from panel respondents who did not contribute in 2016. Then we will work on building longitudinal coverage, requesting samples in 2020 from the 4,000 person subsample with innovative markers and most HCAP-eligible respondents; and from the remaining sample members in 2022 (i.e., those already in repository). Our only proposed change to collection procedures is to reduce the size of the 10 mL SST tubes to make field centrifugation easier. This will reduce the amount of blood requested by from 50.5 mL to 43 mL. If funded, we will have support for assays that are done immediately after collection in order to provide feedback to respondents and for cryopreservation of appropriate samples. We will the HbA1c assay as we have obtained this from DBS in the past and will no longer continue collecting DBS going forward.

The table below compares the 2016 assay protocol with the proposed protocol for the HRS years 29-34. The complete assay list reflects what is being done for the current collection wave whereas the assays in bold are proposed for the renewal period.

Venous Blood Study Assays and Estimated Storage by Collection Tube		
Tube type and blood volume	Assay	Storage
7.5mL SST (Serum) - 1	<b>Total cholesterol, HDL and LDL cholesterol, Triglycerides</b> , High sensitivity CRP (hsCRP), Vitamin D (25 Hydroxy), Ferritin (FRTN), IGF-1 , DHEAs, Cytokine Panel (IL-6, IL-1RA, IL-10, TNF-alpha, sTNFR-I, and TGF beta [activated form]), B-type natriuretic peptide (NT-proBNP)	8 mL Serum
7.5mL SST (Serum) - 2	<b>Comprehensive Metabolic Panel ([CMP]: Albumin, Alk Phos, ALT, AST, Bili, Ca, Cl, CO2, Creat, Gluc, K, Total protein, Na, BUN)</b> , Cystatin C, Homocysteine, Clusterin, BDNF	
7.5mL SST (Serum) - 3	Repository only	
8 mL CPT	<b>Cryopreserved cells</b> / Flow cytometry	8 Aliquots of cryopreserved cells
10 mL EDTA (Whole Blood)	<b>Complete blood count ([CBC]: White blood cell [WBC, leukocyte] count, White blood cell types [WBC differential], Red blood cell [RBC] count, Hematocrit [HCT, packed cell volume, PCV], Hemoglobin [Hgb], Red blood cell indices: mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], and mean corpuscular hemoglobin concentration [MCHC], Platelet [thrombocyte] count, Mean platelet volume [MPV]),</b> CMV seroprevalence, mtDNA copy number, telomere length, DNA methylation, <b>HbA1c**</b>	3.5 mL Plasma Buffy coat Extracted DNA
2.5 mL Paxgene RNA	RNA-seq	Stabilized RNA

\* Assays in bold are included in 2018-2023 protocol

\*\* HbA1c added for 2018-2023