NATIONAL HEALTH AND AGING TRENDS STUDY (NHATS)

Dried Blood Spot (DBS) Based Biomarkers User Guide

FINAL RELEASE

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Contents

Introduction

This document describes the dried blood spot (DBS) collection conducted in Round 7 (2017) of the National Health and Aging Trends Study (NHATS). Four assays were conducted from the DBS samples, and were selected for their association with the biologic risk of disability progression. This technical paper provides details on the collection and assays of these samples and documentation to facilitate analysis of the DBS data.

Eligibility and Response Rates

All Round 7 living sample persons with a completed interview were considered eligible for the DBS collection. However, sample persons whose interviews were being conducted with proxy respondents were not asked to participate in the DBS collection because self-response was required for the DBS consent process. The table below provides numbers and response rates for persons eligible to participate in the DBS collection by type of interview and consent to participate.

Table 1. Round 7 eligible respondents and consent for DBS participation

Type of interview & consent status if asked	Eligible and invited	Eligible and not invited	All Eligible
Self-report, Consented	4903	---	4903
Self-report, Did not consent	362	1*	363
Proxy Interview	---	300	300
Total	5265	301	5566
Response rate	93.1%	---	88.1%

*Considered a completed interview but broke-off prior to question inviting participation in DBS.
Note: Persons living in nursing homes or non-nursing home residential care settings who do not have a sample person interview (n = 287) and proxy respondents to the Last Month of Life interview (n=459) were ineligible.

Consent Procedures

The consent form (see Appendix A) was read to the sample person by the interviewer. The form covered the study purpose, procedures, plans for assays for blood spots collected, plans for storing blood spots, and the risks/discomforts and benefits of the collection. The consent form also included two items regarding consent for future research with stored blood spots, one giving consent for "future research about health, aging, and disability" and one giving consent for "genetic research about health, aging, and disability."
Among persons consenting to the DBS collection:

- 4784 (97.6%) of eligible and consenting respondents also consented to future research
- 4767 (97.2%) of eligible and consenting respondents also consented to genetic research

Data Collection Procedures

Administration of the DBS collection was guided by the Blood Collection (BC) section of the Round 7 CAPI (Computer-Assisted Personal Interview) instrument and the NHATS DBS collection protocol (DBS Job Aid for interviewers; Appendix B). After receiving signed consent from respondents, interviewers followed the steps in the DBS Job Aid. The NHATS DBS collection protocol was developed with the assistance of Dr. Teresa Seeman (NHATS co-investigator) and Dr. Heather McCreath from the David Geffen School of Medicine, UCLA. In Round 6 (2016), the DBS collection protocol was pilot tested by 4 interviewers who collected DBS samples for 48 sample persons. Several refinements to the protocol were made based on the pilot test experience, including the decision to aim for collecting 5 spots on a single card and the use of a small heating pad (hand warmer) to warm the sample person's hand and increase blood flow. The NHATS DBS Job Aid provides detailed steps for interviewers in terms of arranging equipment and supplies, conducting the finger prick procedure to obtain blood spots, and handling of the DBS card following collection (placement in a drying case). Interviewers wrote the date and their NHATS ID on the DBS card and then scanned the DBS kit ID and card ID into CAPI. The number of blood spots that were collected on the DBS Card also was entered into CAPI.

DBS cards were kept overnight by interviewers to allow time to dry before shipping back to the Westat home office. The DBS Shipping Job Aid (Appendix C) provides details on the instructions to interviewers for shipping the cards.

Once cards were received at Westat, they were stored in freezers at -20°c and then batched for shipment to the University of Washington Laboratory. Eight batches of DBS cards were shipped (1st shipment on July 13, 2017; last shipment on December 20, 2017). Individual cards were in small ziplock bags with 1 desiccant and then placed in large freezer bags (25 cards per bag) with additional desiccants. Shipments of freezer bags in containers with ice packs (provided by the University of Washington lab) were sent by FedEx overnight priority delivery for arrival at the University of Washington Laboratory the next day.

Initial and Follow-up Collection

DBS collection was carefully monitored from the start of Round 7 fieldwork in May, 2017. The goal of obtaining 5 spots allowed for the planned assays as well as for sample to store for future research. DBS cards were sent to the University of Washington (UW) Lab (overseen by Dr. Alan Potter, Department of Laboratory Medicine, University of Washington School of Medicine) on a flow basis. As punches were taken from the blood spots for the planned assays, UW provided reports that allowed identification of cases that were not providing sufficient "good" punches (largely due to too few blood spots or drops that were too small or of insufficient quality).

Using the report from the University of Washington Lab, we identified 1,195 persons who had consented to the DBS collection from May through September 5, 2017, but whose blood spots

provided fewer than 6 “good” punches (the minimum needed for the 4 planned assays). Starting in early October, we began a second DBS collection with these individuals and succeeded in obtaining a second DBS card (with up to 5 spots) for 1,004 persons.

In all, 4,691 persons (95.7%) of those who consented had at least 1 card with DBS sample available for assaying.

DBS Assays selected and conducted

The NHATS Data Monitoring Committee and NHATS co-investigators, Dr. Jeremy Walston, Johns Hopkins School of Medicine, and Dr. Teresa Seeman, UCLA, advised on selection of the assays. The four assays selected were those likely to provide information on multiple biologic risks (e.g. heightened inflammation, metabolic dysregulation, and possible immune function) for the onset of impairments that underlie disability. The assays selected are: high-sensitivity C-reactive protein (hsCRP), glycosylated hemoglobin (HbA1c), Cytomegalovirus (CMV) and Interleukin (IL)-6. The assays were conducted at the Department of Laboratory Medicine, University of Washington School of Medicine by Jake Cofferen and Elizabeth Kerschner under the supervision of Dr. Alan Potter. Details of the laboratory procedures for conducting the assays were provided by Dr. Alan Potter (Appendix D). The samples were processed to perform the assays in the following order: IL6, HbA1c, CMV, and hsCRP.

Table 2. Assays with Results by Type of Assay Among Persons Who Consented to DBS participation

Number and Type of Assays	Persons with Results	Persons with No Results	Total who Consented to DBS
Number of Assays with Results			
0	----	255*	255
1	99	---	99
2	103	---	103
3	758	---	758
4	3688	---	3688
Type of Assay			
HbA1c	4401	502	4903
hsCRP	4336	567	4903
CMV	4416	487	4903
IL-6	4178	725	4903
Total	4648	255	4903

*212 persons with no DBS card available and 43 persons with a card that yielded no assay results

Data Files and variables

The DBS analytic file includes all Round 7 participants (n = 6,312). Persons who were ineligible for the DBS collection (n = 746), persons with proxy respondents who were not asked to

participate (n = 300), and those who did not consent (n = 363), are coded -1 (inapplicable) on all variables.

Paradata variables: Indicators are included for: the number (1 or 2) of DBS cards obtained, month the cards were obtained, which card (1, 2, or both) was used for each assay, and the month and year when each assay was conducted. For HbA1c only, there is a batch code indicating whether the assay was conducted before or after a preventive maintenance service. Higher variance in the data was observed in assays conducted in the pre-service period.

Results variables: Two types of results variables are provided for each assay: directly measured analyte concentrations and plasma-equivalent concentrations (blood-equivalent values for HbA1c). The analyte concentrations obtained from DBS samples are direct values (From Dr. Alan Potter: the analyte concentration in dried blood eluted (re-liquefied) using a fixed volume of buffer from a 3.2mm diameter filter paper disc punched from a DBS). Directly measured concentrations are converted into plasma-equivalent concentrations using a linear regression equation obtained from a comparison of analyte concentrations in conventional plasma samples versus analyte concentrations in DBS samples matched to those plasma samples. The plasma-equivalent values are provided to allow evaluation of the DBS assay results against other published metrics (for example, values obtained from the National Health and Examination Survey). Distributions of direct analyte and plasma equivalent values for each assay are in Appendix E.

Variables from notes related to the DBS quality and assaying process: Three variables are included that provide information about the quality of the DBS samples and the assaying process: result notes, punch notes and assay notes. Assay notes and punch notes are made by the technician performing the assay (assay notes) or sample processing (punch notes). Result notes indicate the data manager's identification of a punch or assay note that affected the assay result. Analysts may choose to consider this information when results are missing or do sensitivity analyses, for example excluding cases with codes indicating data quality or assaying problems, to see if results change.

Values of Result and Assay note variables differ by type of assay. With the exception of HbA1c, there is one result note variable and one assay note variable for each assay.

Table 3. Result and Assay Note Variables and Values by Type of Assay

Type of Note and Assay	Variable name	Values
Result Note		
Il6	dbs7rnoteil6	1 Blank punch, no result 2 Below lower limit of detection (LLoD) 3 Above analytical measurement range (AMR) 4 Issue during assay, no result

HbA1c	dbs7rnotea1c	1 Blank punch, no result 2 Interfering peak, no result 3 No A1c peak, no result 4 %HbA1c adjusted due to false low level of HbS 5 Bad chromatogram: area <1.5M, no result 6 Bad chromatogram: aberrant A1c integration, no result 7 HPLC instrument error, no result 8 Non-A hemoglobin variant >60%; no result
CMV	dbs7rnotecmv	1 Blank punch, no result 2 Above analytical measurement range (AMR), no result 3 Equivocal, no result 4 Negative, no result
hsCRP	dbs7rnotehscrp	1 Blank punch, no result 2 Issue during assay, no result 3 Below lower limit of detection (LLoD), no result 4 Below limit of quantification (LoQ); CV>20% 5 Above analytical measurement range (AMR), no result 6 Punch did not elute, no result
Assay Note		
Il6	dbs7anoteil6	1 Blank punch, no result 2 Below lower limit of detection (LLoD) 3 Above analytical measurement range (AMR) 4 Issue during assay, no result
HbA1c	dbs7intpeaka1c	-1 Not Applicable 1 Interfering peak A1c, no result
	dbs7nopeaka1c	-1 Not Applicable 1 No A1c peak, no result
	dbs7adjusta1c	-1 Not Applicable 1 %HbA1c adjusted due to false low level of HbS
	dbs7badinta1c	-1 Not Applicable 1 Bad chromatogram integration >1.8min A1c
	dbs7smareaa1c	-1 Not Applicable 1 Bad chromatogram area <1.5M A1c, no result
	dbs7aberranta1c	-1 Not Applicable 1 Bad chromatogram aberrant A1c integration, no result
	dbs7rampinga1c	-1 Not Applicable 1 Ramping baseline A1c
	dbs7instrmnta1c	-1 Not Applicable 1 Instrument error A1C, no result
	dbs7nonhgvara1c	-1 Not Applicable 1 Non-HbA hemoglobin variant >60% A1c, no result
CMV	dbs7anotecmv	1 Blank punch, no result 2 Above analytical measurement range (AMR), no result 3 Equivocal, no result 4 Negative, no result
hsCRP	dbs7anotehscrp	1 Blank punch, no result

		2 Issue during assay, no result 3 Below lower limit of detection (LLoD), no result 4 Below limit of quantification (LoQ); CV>20% 5 Above analytical measurement range (AMR), no result 6 Punch did not elute, no result

Each Punch Note is represented by a variable coded 1 (yes) or -1 (inapplicable).

Punch Notes	**Variable Names**
Blank Punch	dbs7blank###
Multiple Drops Per Printed Circle	dbs7mdrops###
Smeared Drops/Inconsistent Absorption	dbs7smear###
Small Spot, <80%	dbs7small###
Non-circular DBS	dbs7notcirc###
Overlapping Blood Spots	dbs7overlap###
Contaminated Sample	dbs7contamilil6
Physical Damage to Sample	dbs7damage$$$
Abnormal Color	dbs7abcolor###
Does not meet quality criteria	dbs7quality###
Drop outside printed circle	dbs7outside###
No DBS Remains	dbs7norem###

= il6; hba1c; cmv; hscrp
$$$= a1c; cmv; hscrp

Analytic Weights

The dried blood spot weights are used in analyses of dried blood spot assay results. Weighted estimates are generalizable to the US population ages 67 or older in 2017 (Round 7).

All living NHATS participants who completed a Round 7 interview were considered eligible. As shown in Table 1 above, eligible cases included both self-responding participants and proxy interviews, but the latter were not invited to participate. Nonresponse adjustments were made for three groups of eligible persons for whom no DBS results were obtained: proxy interview cases (n=300; not asked to participate; see Table 1 above); self-responding NHATS participants who did not consent to participate in the DBS collection (n = 363; see Table 1 above); and self-responding participants who consented but for whom no valid assay results could be obtained (n = 255; see Table 2 above).

The computation of the DBS weights began with the final NHATS Round 7 Analytic Weight, and included separate weighting class adjustments to account for the 3 types of nonresponse listed above (Appendix F). The candidate variables for forming the adjustment cells for each

adjustment included the participant's age, educational attainment, race/ethnicity, gender, and residential setting in Round 7.

The final DBS weight is provided as the variable W7DBSFINWGT0 (n = 4,648). Replicate weights are also provided (the variables W7DBSFINWGT1 - W7DBSFINWGT56 in the DBS file), and were computed using the full set of adjustments described here; these weights may be used with software that uses replication methods to estimate variances of estimates from complex sample surveys. The replication approach that was used is the modified balanced repeated replication (BRR) method suggested by Fay (Judkins 1990). Fay's method perturbs the weights by ±100 (1-K) percent where K is referred to as "Fay's factor." The perturbation factor for standard BRR is 100 percent, or K=0. For the NHATS DBS weight, K = 0.3 was used. The variables W7VARSTRAT and W7VARUNIT are also provided in the DBS file for use in software that uses the Taylor series linearization method to estimate variances of estimates from complex sample surveys. (These are the "stratum" and "cluster" variables, respectively.)

Data Documentation

The Instruments and Crosswalk are publicly available at www.nhats.org.The round 7 instrument, including the Blood Collection section, is found at https://www.nhats.org/scripts/dataCollInstrR7.htm. A crosswalk between the instrument and codebook is available at https://www.nhats.org/scripts/dataCollVariableQs.htm.

Obtaining DBS Data

DBS files are designated as Sensitive for purposes of data release. To obtain the data files and codebook, go to Sensitive and Restricted Data on the NHATS website. Download the document titled "Obtaining Sensitive Data from the National Health and Aging Trends Study" and follow the instructions.

Appendix A. NHATS DBS Informed Consent Document

Approval Date: December 8, 2016
Approved Consent Version No.: 2
PI Name: Judith Kasper
IRB No. 00002083

JOHNS HOPKINS BLOOMBERG SCHOOL OF PUBLIC HEALTH

INFORMED CONSENT DOCUMENT FOR
BLOOD SPOT DATA COLLECTION IN THE
NATIONAL HEALTH AND AGING TRENDS STUDY

Study Title: National Health and Aging Trends Study – Blood Spot Collection

Principal Investigator: Judith Kasper, Ph.D.

IRB No.: #2083

PI Version Date: December 1, 2016

What you should know about this study

- You are being asked to join the blood spot study because you are in the National Health and Aging Trends Study.
- This consent form explains the research study and your part in the study.
- Please read it carefully and take as much time as you need.
- You are a volunteer. You can choose not to be in the blood spot study. You will still be in the National Health and Aging Trends Study if you do not participate in the blood spot study.

Purpose of research project

The purpose of this study is to collect blood spots to get other markers of health and functioning of older people. We will do several tests on the blood spots we collect. These tests will provide measures of how well glucose levels are controlled (hemoglobin A1C) and markers of inflammation (levels of IL-6, C-reactive protein, and CMV). Research has shown these are related to health outcomes, but tests based on blood spots like we are collecting are not used in medical treatment at this time.

We also will store some blood spots for future research on health and functioning of older people. We will ask you separately about storing some blood spots for this purpose.

Procedures

We will be pricking your finger to collect a few drops of blood. We will first warm your hand and then prick your finger with a sterile instrument. We will collect the drops of blood on a small card. The card will not have your name or other personal information on it. It will take about 5 to 10 minutes to complete the blood spot collection.

Some blood spots will be used to do the blood glucose and inflammation tests at a laboratory at the University of Washington in Seattle, Washington. Any remaining blood spots on the card will be stored for future research.

Future Research Use of Stored Blood Spots

The blood spots that are stored for future research will be used to learn about health, aging, and disability. We will keep the blood spots indefinitely. If at any time you want to have your blood spots removed from storage, you can send a written request to the principal investigator listed at the end of this consent form asking that your blood spots be removed from storage and destroyed. We will not be able to get back test results that have already been shared for research.

Future research on your stored blood spots may include genetic research. Genetic information about you and other participants in the blood spot study may be sent to the National Institutes of Health (NIH) Genome-Wide Association Studies (GWAS) repository. All data sent to the NIH repository is coded and de-identified to protect confidentiality. The National Institutes of Health GWAS repository stores genetic information from many studies and shares that information with researchers who are approved by the National Institutes of Health. The researchers who receive data must promise to keep the data confidential and to use it only for the research purposes approved by NIH.

Risks/discomforts

The risks from taking part in the blood spot study are low. Pricking your finger may be briefly uncomfortable.

All test results done from the blood spots being collected will be kept private and safe like the rest of the data collected in the National Health and Aging Trends Study. We use a unique number code to label your information, and your name and address are kept separate from the number code. We will store the blood spot test results in a secure computer database in a secure facility. The blood spots that are stored for future research will have a unique number code but will have no other personal information.

Benefits

There is no direct benefit to you for taking part in the blood spot study. The blood spot collection provides new information that may help researchers understand changes in health and functioning in older people.

Payment

You will receive $50 for taking part in the blood spot study.

Who do I call if I have questions, problems, or wish to cancel my consent?

- Call the principal investigator, Dr. Judith Kasper, toll-free at 1-888-364-8271 if you have questions or problems as a result of being in this study. To cancel your consent, write to:

Dr. Judith Kasper
Department of Health Policy and Management
Johns Hopkins Bloomberg School of Public Health
Room 641, 624 N. Broadway
Baltimore, MD 21205

- Call or contact the Johns Hopkins Bloomberg School of Public Health IRB Office if you have questions about your rights as a study participant. Contact the IRB if you feel you have not been treated fairly or if you have other concerns. The IRB contact information is:

Address:	IRB Office Johns Hopkins Bloomberg School of Public Health 615 N. Wolfe Street, Suite E1100 Baltimore, MD 21205
Telephone:	410-955-3193
Toll Free:	1-888-262-3242
Fax:	410-502-0584
E-mail:	JHSPH.irboffice@jhu.edu

Choice for Future Research Use of Stored Blood Spots

Please mark an X in the YES or NO box for both statements about future research use.

☐ YES ☐ NO My blood spots may be stored and used for future research to learn about health, aging, and disability.

☐ YES ☐ NO My blood spots may be used for genetic research to learn about health, aging, and disability.

What does your signature on this consent form mean?

Your signature on this form means:

- You have been informed about this study's purpose, procedures, possible benefits, and risks.
- You have been given the chance to ask questions before you sign.
- You have voluntarily agreed to be in this study.
- You have indicated whether your stored blood spots may be used for future research.

Print Study Participant Name	Study Participant Signature	Date

Print Interviewer Name	Interviewer Signature	Date

SP ID ☐☐☐☐☐☐☐☐

Give yellow copy to the participant and keep white original copy in study records

Appendix B. NHATS DBS Job Aid

NHATS Dried Blood Spot (DBS) Job Aid

ORGANIZE SUPPLIES

- DBS Kit
- Bagged gloves
- Sharps container
- Drying case
- Hand sanitizer
- Hand warmer
- Scanner
- Pen
- Stopwatch
- Trash bag
- Extra supplies
- SPID label

1. **GET OUT HAND WARMER**

   I'll warm up your hand to aid in the collection. Please let me know which hand you prefer we use for this activity.

   - **PLUG IN WARMER AND SET ON HIGH** and place on folded warmer cover
   - **ADJUST SEATING POSITIONS**

2. **USE HAND SANITIZER**

   Now we're going to clean our hands with sanitizer. Please follow along with me.

   - **APPLY HAND SANITIZER** to your hands and SP's hands.

   Rub this into your hands and then shake below your waist to dry them.

   - **DEMONSTRATE** rubbing hands with sanitizer and shaking below waist to dry.

3. **WARM SP'S HAND**

   I'd like to begin warming up your hand.

   - **PLACE SAFETY DRAPE OVER SP'S LAP**
   - **WRAP WARMER IN WARMER COVER**
   - **HAVE SP PLACE HAND WITH WARMER IN LAP WITH PALM FACE DOWN**
   - **START STOPWATCH**

4. **OPEN SAFETY DRAPE AND PLACE ON TABLE**

   PLACE AND ORGANIZE SUPPLIES ON THE SAFETY DRAPE:

   - 2 gauze pads
   - Alcohol prep pad
   - Bandage
   - Lancet
   - DBS card (place empty bag back into kit bag)
   - Open sharps container

5. **WRITE DATE AND NHATS ID ON DBS CARD**

   Now I'm going to scan the collection kit ID into my computer, then we'll get started.

6. **RETURN TO CAPI** – follow instructions to scan kit ID and DBS card ID

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NHATS DBS Job Aid (continued)

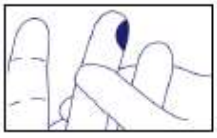
7. **GET INTO POSITION AND PUT ON GLOVES**

8. **OPEN COLLECTION SUPPLIES:** 2 gauze pads, bandage, alcohol prep pad

9. **AFTER 5 MINUTES REMOVE HAND WARMER**
   Would you prefer to use your middle or ring finger for this activity?

10. **CLEAN SP'S FINGER** with alcohol prep pad – then **OPEN DBS CARD FLAP**
    - **PLACE SAFETY DRAPE, DBS CARD, AND 1 GAUZE PAD ON YOUR LAP**
    - **POSITION SP'S HAND OVER SAFETY DRAPE ON LAP**

11. **POSITION LANCET ON SIDE OF THE PAD OF THE SELECTED FINGER CLOSEST TO PINKY**
    Place the lancet perpendicular to the SP's fingerprint on fleshy part of the finger
    I'm going to press down and you will feel a pinch.



12. **FIRMLY PRICK THE FINGER**

13. **IMMEDIATELY PUT LANCET INTO SHARPS CONTAINER**

14. **USE GAUZE TO WIPE AWAY FIRST FULL BLOOD DROP**

15. **COLLECT 5 DROPS ON DBS CARD** - starting with leftmost circle

16. **PLACE NEW GAUZE ON FINGER** - ask SP to hold gauze with fingers pointed toward ceiling for about 30 seconds or until bleeding has mostly stopped

17. **APPLY BANDAGE TO COLLECTION SITE**

18. **PLACE THE DBS CARD IN EMPTY DRYING CASE ON A FLAT SURFACE**
    Leave card flap and drying case flap open to dry for remainder of interview

19. **CLEAN UP COLLECTION MATERIALS**
    - Place all non-sharps trash in safety drape, roll up, and place in trash bag
    - Remove gloves according to procedures and place in trash bag

20. **USE HAND SANITIZER**

21. **PACK UP** all DBS equipment and supplies except for drying case with DBS card
    - Turn off stop watch and hand warmer

22. **RETURN TO CAPI AND RECORD RESULTS**

23. At end of interview, place an SPID label on the kit bag, snap close drying case with DBS card, place drying case back in DBS Kit bag, and pack in DBS equipment bag

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Appendix C. NHATS DBS Shipping Job Aid

NHATS Dried Blood Spot (DBS) Shipping Job Aid

ORGANIZE SUPPLIES

- Gloves
- NHATS ID mini-label
- Drying case with card inside
- DBS Kit bag
- DBS Card bag
- One desiccant
- Hand sanitizer
- Fedex shipper pack
- Fedex preprinted shipping label
- Alcohol prep pad
- Permanent marker

1. Put on a new pair of gloves.
2. Remove DBS card from the drying case.
3. Fold the top card flap down so it sits underneath the barcode labeled portion of the card.
4. Confirm your NHATS ID and data collection date are filled out completely.
5. Put your NHATS ID mini-label on back of the card above the biohazard symbol.
6. Place the card in the individual resealable plastic bag it came in.
7. Add 1 desiccant to the individual resealable plastic bag with DBS card; seal the bag.
8. Place the small resealable plastic bag in the larger DBS kit bag; seal the DBS kit bag.

9. Clean the inside and outside of the drying case with an alcohol prep pad and allow it to dry out so it is ready for re-use.
10. Remove and discard gloves.

11. Wash your hands with soap and water.
12. Insert the case folder and DBS kit bag into a FedEx shipper pack.

13. On the online IMS shipment link, mark the appropriate items that you are shipping back such as the DBS consent signature page, DBS kit bag with card, and incentive receipt form.
14. Write your NHATS ID on the shipping pack with a permanent maker.

15. Ship the case folder back that day using a preprinted shipping label to the NHATS field room (FedEx Priority Overnight) unless weekend or holiday

******All DBS cards must be shipped to the Home Office regardless of whether blood was collected******

Appendix D. Procedures for NHATS DBS Assays conducted by the Department of Laboratory Medicine, University of Washington School of Medicine (Narrative provided by Dr. Alan Potter)

Sample Storage and Punches for Assays

DBS study samples and quality control (QC) samples were sealed with desiccant packs in Ziploc bags and stored at -70°c in freezers at the University of Washington Department of Laboratory Medicine, Seattle, WA (UWLM). For the analyses, a single 3.2mm (1/8in) diameter disc was punched from each DBS (for HbA1, hsCRP, CMV; four discs for IL6) into a 96-well microtiter plate well using a BSD700 Semi-Automated Dried Sample Puncher (BSD Robotics, Brisbane, Australia). Microtiter plates were then assayed immediately or were sealed and stored at -70°c.

Il6

The DBS high-sensitivity interleukin 6 (**IL6**) assay is a sandwich ELISA. Assay Diluent (R&D Systems, Minneapolis, MN) is added to the four DBS discs in each plate well and the plate is then sealed, shaken for 1hr on a microplate shaker (Delfia, PerkinElmer, Waltham, MA) and held for 16hr at 4°c to reliquefy the dried blood and elute IL6. Each well of the plate is pre-coated with an anti-IL6 mAb that binds IL6 in the eluate (solid phase immobilization). The plate is shaken at RT for 45min and then washed. Conjugate Reagent (R&D) containing anti-IL6 Ab coupled to biotin (enzyme-linked antibody) is then added, resulting in IL6 being sandwiched between the solid phase and enzyme-linked Ab. The plate is shaken at RT for 1hr and then washed. Streptavidin Polymer-HRP Solution (peroxidase; R&D) is added and the plate shaken at RT for 30min to permit biotin-streptavidin binding. Substrate Solution (R&D) containing tetramethylbenzidine (TMB) and H_2O_2 is then added and the plate held at RT for 30min; H_2O_2, cleaved by the peroxidase, reacts with TMB to produce a colored solution. The reaction is stopped by addition of Stop Solution (R&D). The OD of each plate well, read at 450nm by a microtiter plate reader (Synergy H1, BioTek, Winooski, VT), is directly proportional to the IL6 concentration. A calibration curve, constructed by plotting the OD values of the calibrators against the assigned IL6 concentrations (Gen 5, BioTek), is used to convert the OD value of each sample into a DBS direct IL6 concentration (pg/mL). Assay acceptability is determined by comparing the IL6 concentrations of liquid (R&D) and DBS (UWLM) QC samples against the established values. Assay calibrators were provided by the vendor (R&D). DBS QC samples were constructed by UWLM from human plasma mixed with a constant volume of washed human erythrocytes and aliquoted onto Whatman No. 903 filter paper (GE Healthcare Bio-Sciences, Pittsburgh, PA). The IL6 concentration of each QC sample was determined by ELISA (R&D). The IL6 concentrations of samples analyzed by the DBS assay correlated with the IL6 concentrations of DBS-matched plasma samples (Pearson r = 0.93) and were linearly related (DBS plasma-equivalent IL6 concentration = -2.225 + DBS direct IL6 concentration X 10.58).

HbA1c

The hemoglobin A1c (**HbA1c**) assay employs a Variant II Hemoglobin Testing System (Bio-Rad Laboratories, Hercules, CA), an automated ion-exchange high-performance liquid chromatograph (IE-HPLC), to measure the percentage of glycosylated hemoglobin (%HbA1c) in each DBS. HbA1c buffer (Diluent Reagent, Bio-Rad) is added to each microtiter plate well, and the plate is then shaken on a microplate shaker (Delfia) at room temperature (RT) for 1hr to elute (reliquefy) the dried blood. The eluate is transferred to a vial containing Diluent Reagent, shaken for 30sec and then analyzed. The Variant II applies a buffer gradient of increasing ionic strength to separate hemoglobin species based on ionic interactions with the cation exchange cartridge resin. Hemoglobins are identified by a characteristic time of passage through a filter photometer. The chromatogram curves are integrated to determine the HbA1c and total HbA areas, and %HbA1c is determined from the HbA1c: total HbA ratio adjusted by the calibration curve parameters (Clinical Data Management Software, Bio-Rad). DBS QC samples were constructed at UWLM by aliquoting blood with known %HbA1c values onto Whatman No. 903 filter paper (GE). Acceptability of each assay is determined by comparing the %HbA1c values of liquid QC samples (Bio-Rad) and DBS QC samples (UWLM) against the established values. Acceptability of data from each DBS is determined by examining the chromatogram for proper form, absence of interfering peaks, acceptable total area, and %HbA1c value within the analytical measurement range (3.1% to 18.5% per established limits (Bio-Rad). The %HbA1c values of 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 = -1.319 + DBS direct %HbA1c value X 1.284).

One set of samples was assayed from January 12th to March 30th. The Variant then received a preventative maintenance service (PM) before the second set of samples was assayed from June 19th to September 13th. Although the performance of the Variant II was acceptable throughout Jan-Mar and Jun-Sep (e.g., values of QC samples were as expected) the performance improved (decreased variance) after the PM. When evaluating the data we suggest that a batch variable be used to control for the higher variance in the data from the pre-PM period vs. in the post-PM period.

CMV

The DBS anti-cytomegalovirus IgG antibody (**CMV**) assay is a sandwich ELISA. Sample Diluent (Diamedix Corp, Miami Lakes, FL) is added to each microtiter well containing a DBS punch and the plate is then shaken at RT for 1hr on a microplate shaker (Delfia) to reliquefy the dried blood and elute CMV. The eluate is transferred to a microtiter plate (Diamedix) pre-coated with a CMV antigen that is recognized and bound by CMV (solid phase immobilization). The plate is incubated at 37°c for 1hr and then washed. Enzyme Conjugate (Diamedix) containing anti-IgG Ab coupled to peroxidase (enzyme-linked antibody) is then added, resulting in CMV in the eluate being sandwiched between the solid phase and conjugate. The plate is incubated at 37°c

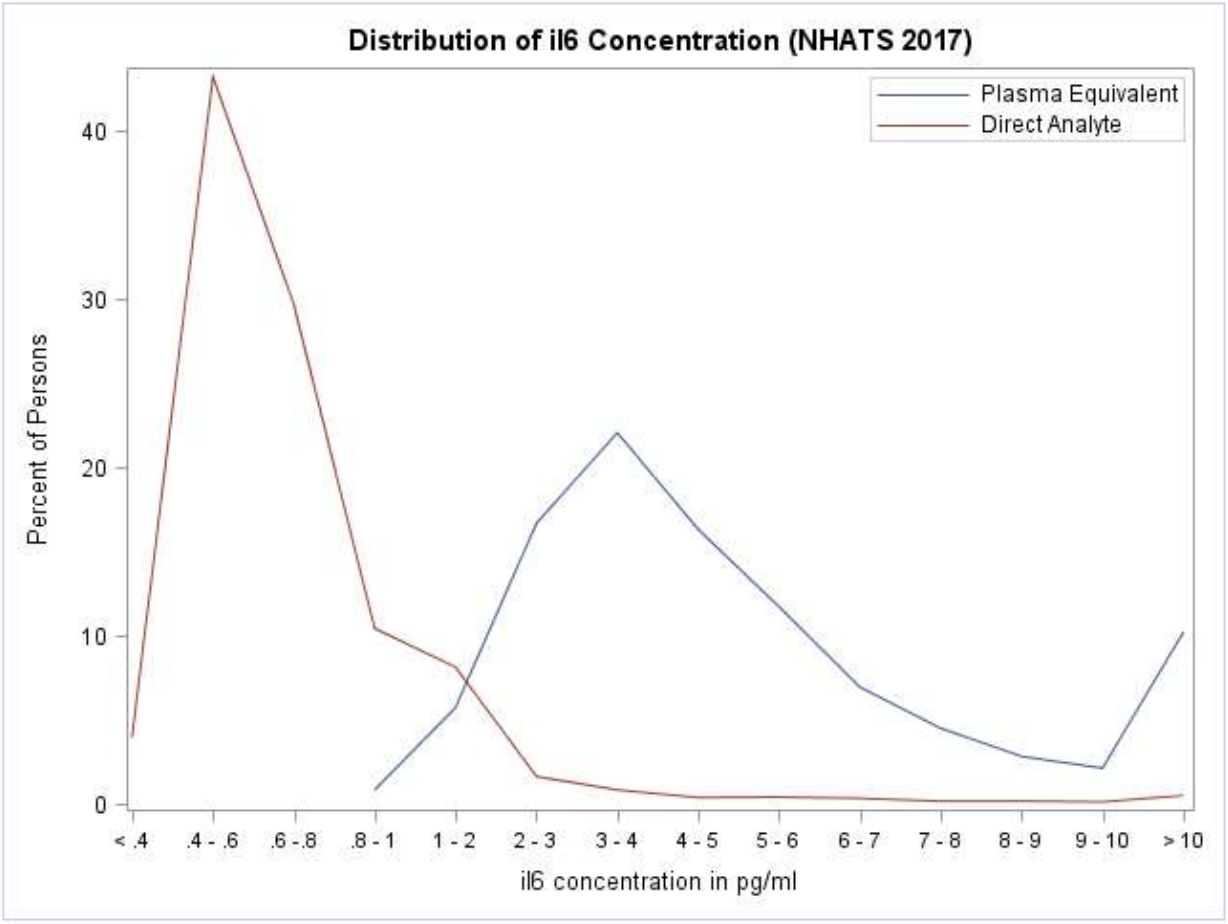
for 1hr and then washed. Substrate Solution containing TMB and H_2O_2 (Diamedix) is added and the plate incubated at 37°c for 20min; H_2O_2, cleaved by the peroxidase, reacts with TMB to produce a colored solution. The reaction is stopped by addition of Stop Solution (Diamedix). The OD of each plate well, read at 450nm by a microtiter plate reader (Synergy HT, BioTek), is directly proportional to the CMV concentration. A calibration curve, constructed by plotting the OD values of the calibrators against the assigned CMV concentrations (Gen 5), is used to convert the OD value of each sample into a DBS direct CMV concentration (Diamedix assay units, AU/mL). Acceptability of the assay is determined by comparing the CMV concentrations of liquid (Bio-Rad) and DBS (UWLM) QC samples against the established values. Assay calibrators are provided by the vendor (Diamedix). DBS QC samples were constructed from human plasma samples, either CMV positive or CMV negative (UWLM). Each QC sample solution was mixed with a constant volume of washed human erythrocytes (UWLM), pipetted in aliquots onto Whatman No. 903 filter paper (GE). The CMV concentration of each QC sample was determined by ELISA (Diamedix). The CMV concentrations of CMV-positive DBS samples analyzed by the DBS assay correlated with the CMV concentrations of DBS-matched plasma samples (Pearson r = 0.98) and were linearly related (plasma-equivalent CMV concentration = 13.97 + DBS direct CMV concentration X 1.436).

hsCRP

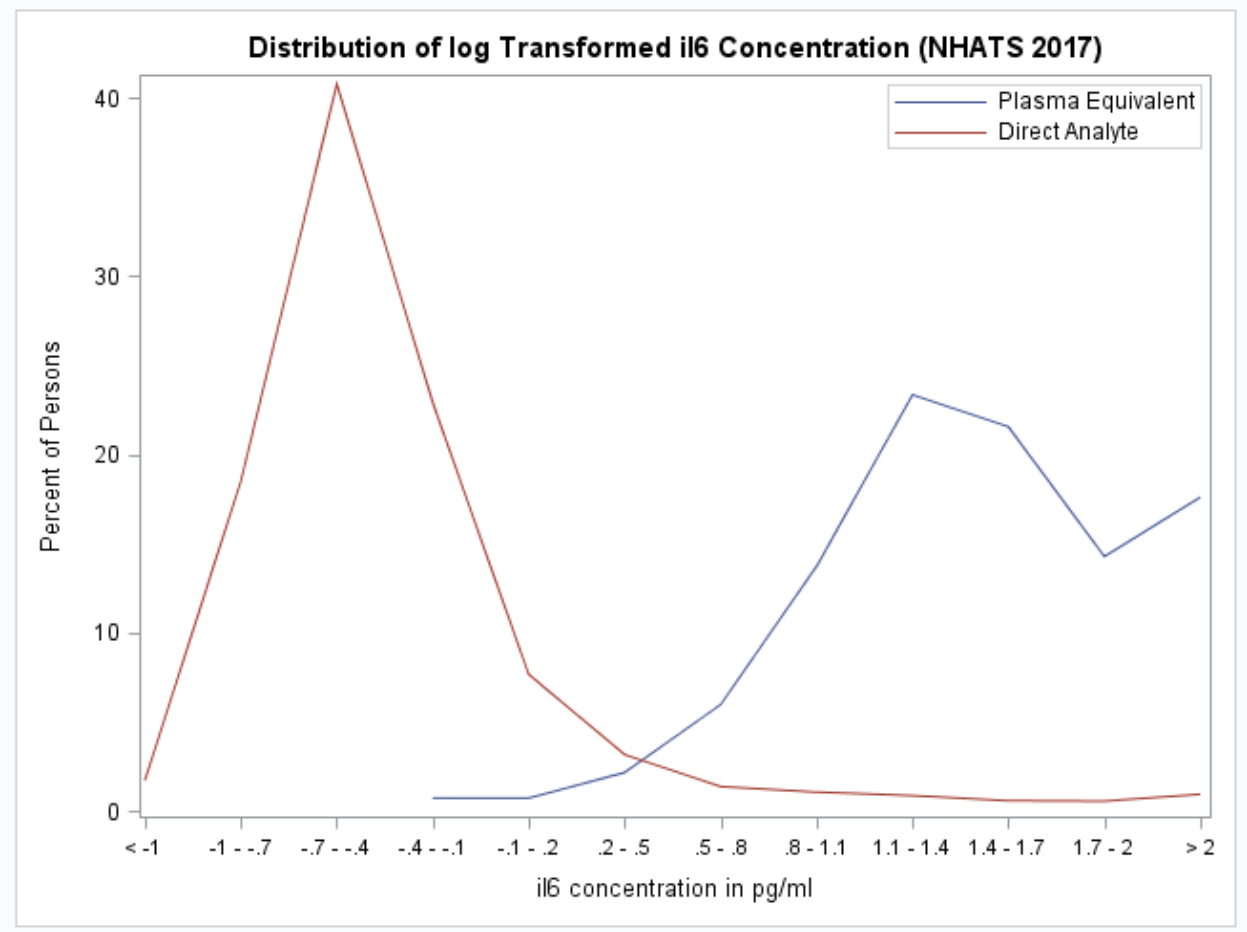
The DBS high-sensitivity C-reactive protein (**hsCRP**) assay is a sandwich ELISA. Sample Diluent (Percipio, Manhattan Beach, CA) is added to each microtiter well containing a DBS punch; the plate is then shaken on a microplate shaker (Delfia) at RT for 1hr to reliquefy the dried blood and elute CRP. Eluate is transferred to a microtiter plate (Percipio) pre-coated with an anti-CRP monoclonal antibody (mAb) that recognizes and binds CRP (solid phase immobilization). Enzyme Conjugate (Percipio) containing anti-CRP Ab coupled to peroxidase (enzyme-linked antibody) is then added, resulting in CRP in the DBS eluate being sandwiched between the solid phase and enzyme-linked antibodies. The plate is shaken at RT for 45min and then washed. TMB Reagent containing H_2O_2 (Percipio) is added and the plate incubated at RT for 20min; H_2O_2, cleaved by the peroxidase, reacts with TMB to produce a colored solution. The reaction is stopped by addition of Stop Solution (Percipio). The OD of each plate well, read at 450nm by a microtiter plate reader (Synergy HT), is directly proportional to the CRP concentration. A calibration curve, constructed by plotting the OD values of the calibrators against the established concentrations (Gen 5), is used to convert each OD value into a DBS direct CRP concentration (mg/L). Assay acceptability is determined by comparing the CRP concentrations of liquid (Bio-Rad) and DBS (UWLM) QC samples against the established CRP concentrations. Assay calibrators were provided by the vendor (Percipio). DBS QC samples were constructed from pooled human plasma, either undiluted (high CRP) or diluted with negligible CRP plasma (low CRP). Each QC solution was mixed with a constant volume of washed human erythrocytes and aliquoted onto Whatman No. 903 filter paper (GE). The CRP concentration of each QC sample solution was determined by analysis on an AU680 Clinical Analyzer (Beckman Coulter, Miami, FL). The CRP concentrations of samples analyzed by the DBS assay correlated with the

CRP concentrations of DBS-matched plasma samples (Pearson r = 0.99) and were linearly related (DBS plasma-equivalent CRP concentration = 0.0457 + DBS direct CRP concentration X 0.7836).

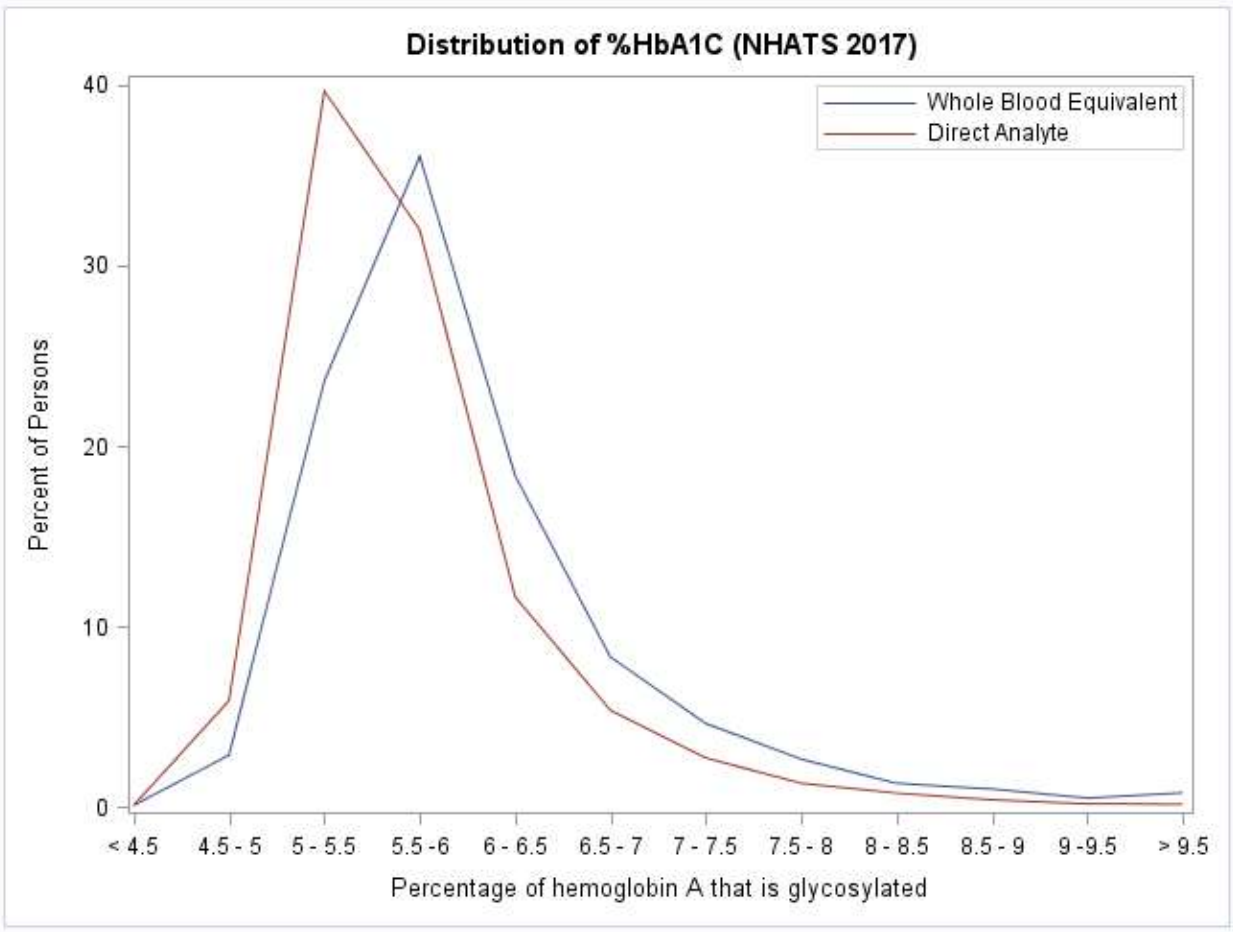
Appendix E. Frequency Distribution of Assay Results



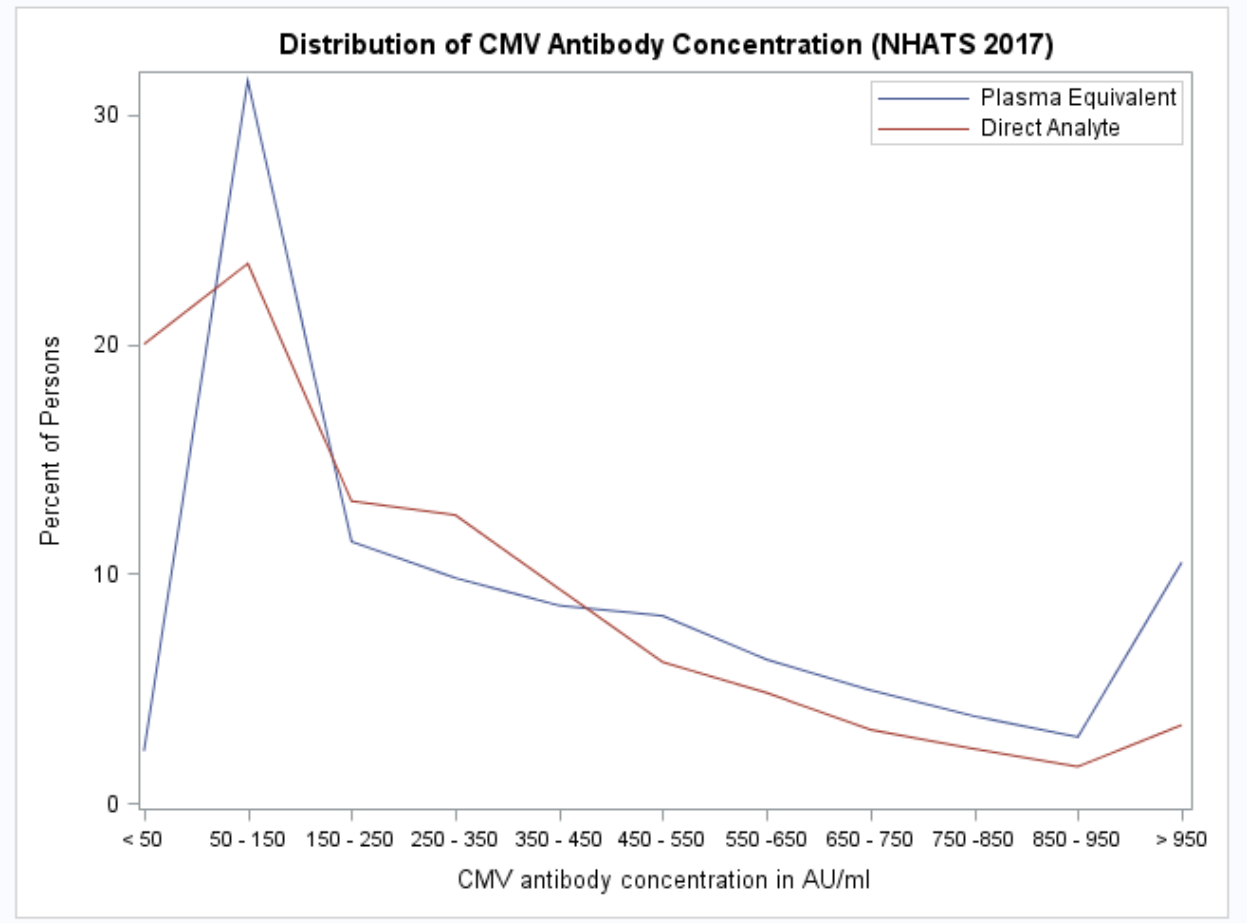
Assay Result	Direct Analyte	Plasma Equivalent
N	4178	4178
Mean	0.851	7.09
Median	0.614	4.252
Interquartile Range	0.259	3.099
Minimum (23 cases capped at minimum)	< 0.338	< 0.853
Maximum (21 cases capped at maximum)	> 10.000	> 116.000



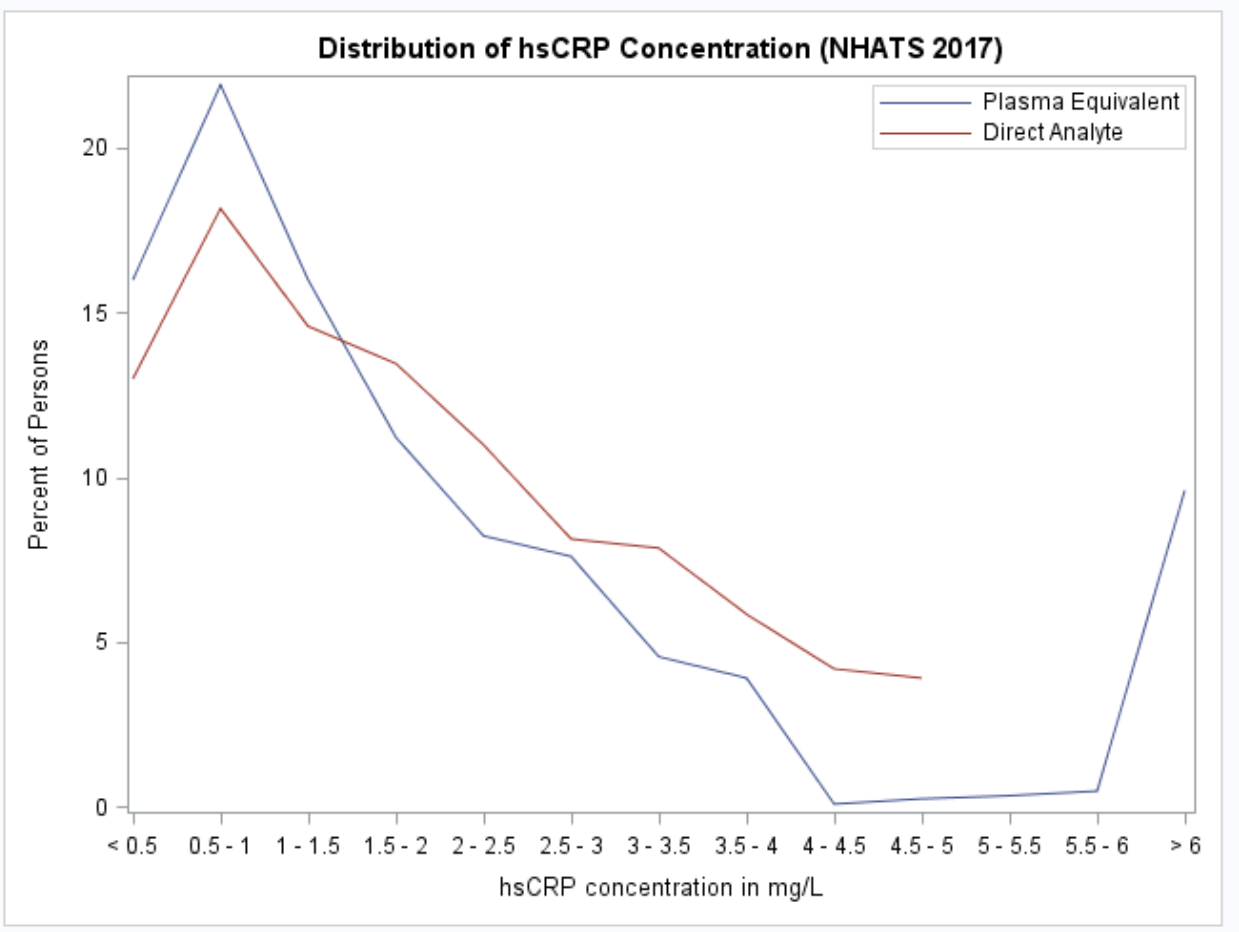
Assay Result	Direct Analyte	Plasma Equivalent
N	4178	4178
Mean	-0.374	1.546
Median	-0.488	1.447
Interquartile Range	0.407	0.696
Minimum(23 cases capped at minimum)	< - 1.106	< - 0.159
Maximum (21 cases capped at maximum)	> 2.303	> 4.754



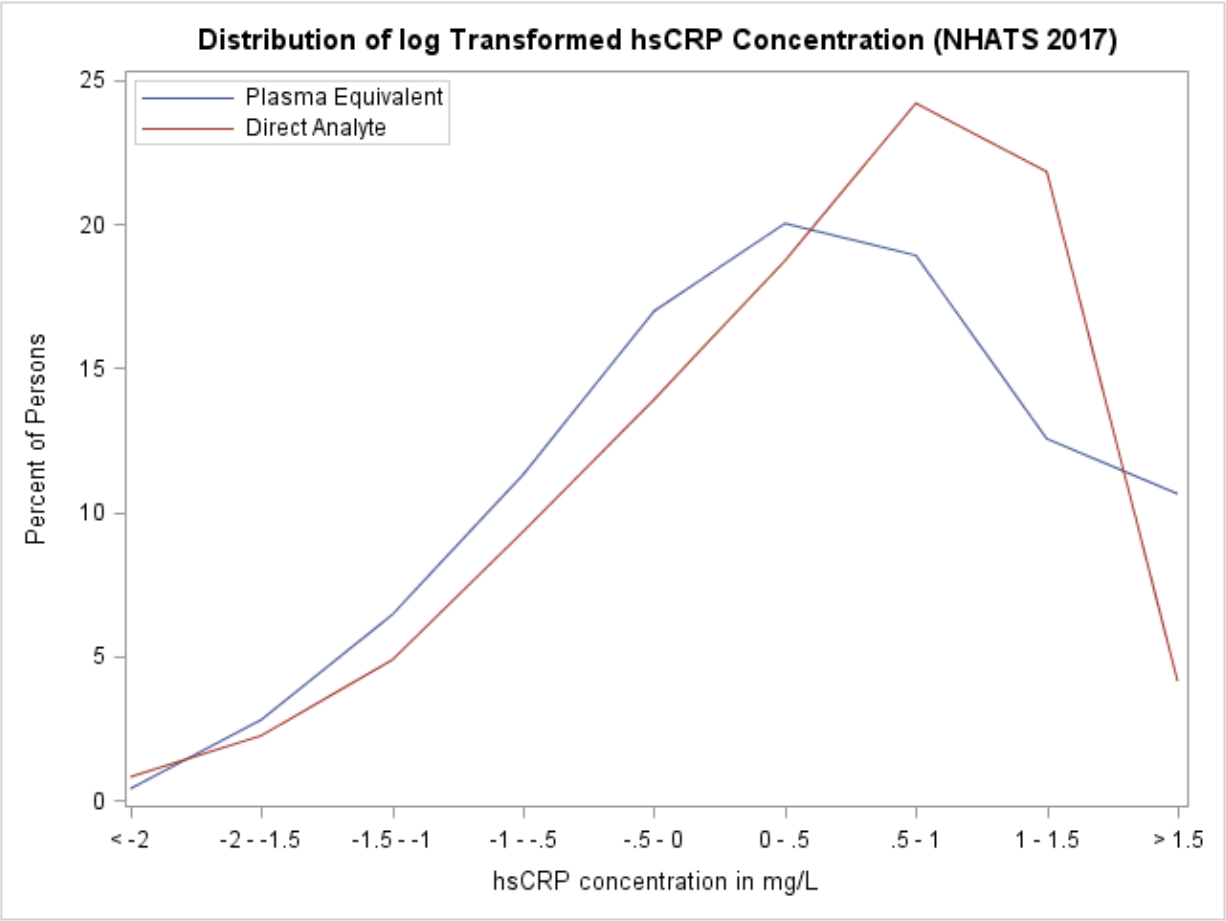
Assay Result	Direct Analyte	Whole Blood Equivalent
N	4401	4401
Mean	5.7	6.0
Median	5.5	5.7
Interquartile Range	0.7	0.9
Minimum	4.2	4.1
Maximum	13.4	15.9



Assay Result	Direct Analyte	Plasma Equivalent
N	4416	4416
Mean	283.4	421.0
Median	198	298
Interquartile Range	340	489
Minimum	20	43
Maximum	1585	2290



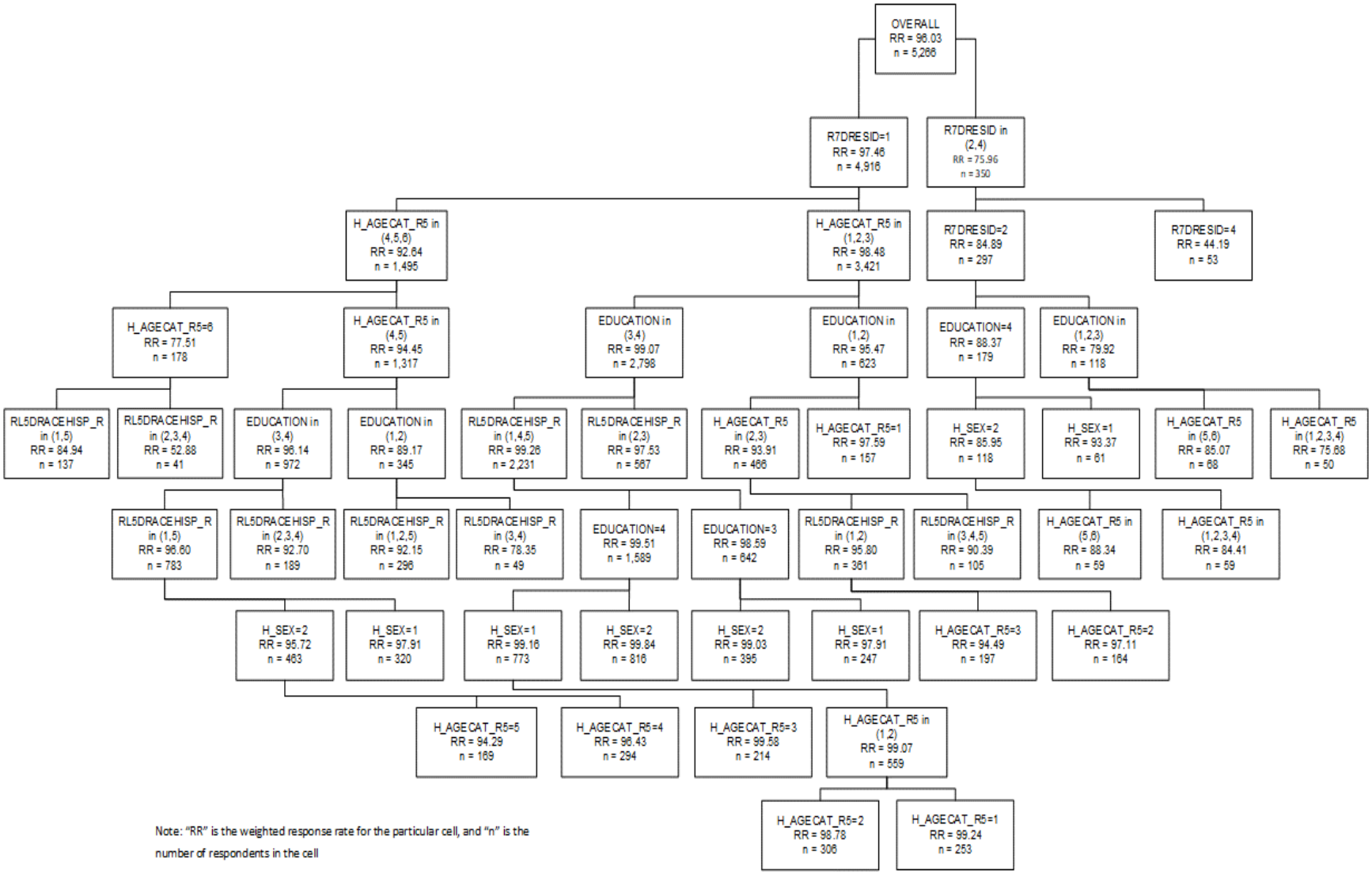
Assay Result	Direct Analyte	Plasma Equivalent
N	4336	4336
Mean	1.894	2.376
Median	1.653	1.353
Interquartile Range	1.973	1.915
Minimum	0.075	0.106
Maximum	4.998	19.662



Assay Result	Direct Analyte	Plasma Equivalent
N	4336	4336
Mean	0.349	0.323
Median	0.503	0.302
Interquartile Range	1.228	1.333
Minimum	- 2.590	- 2.244
Maximum	1.609	2.979

Appendix F. NHATS Round 7 DBS Weights Nonresponse Adjustment Figures

Figure 1. Round 7 2015 Cohort DBS Weight Nonresponse Adjustment Cells – adjusting for SP interview completed by proxy



Note: "RR" is the weighted response rate for the particular cell, and "n" is the number of respondents in the cell

Figure 2. Round 7 2015 Cohort DBS Weight Nonresponse Adjustment Cells – adjusting for no consent for DBS

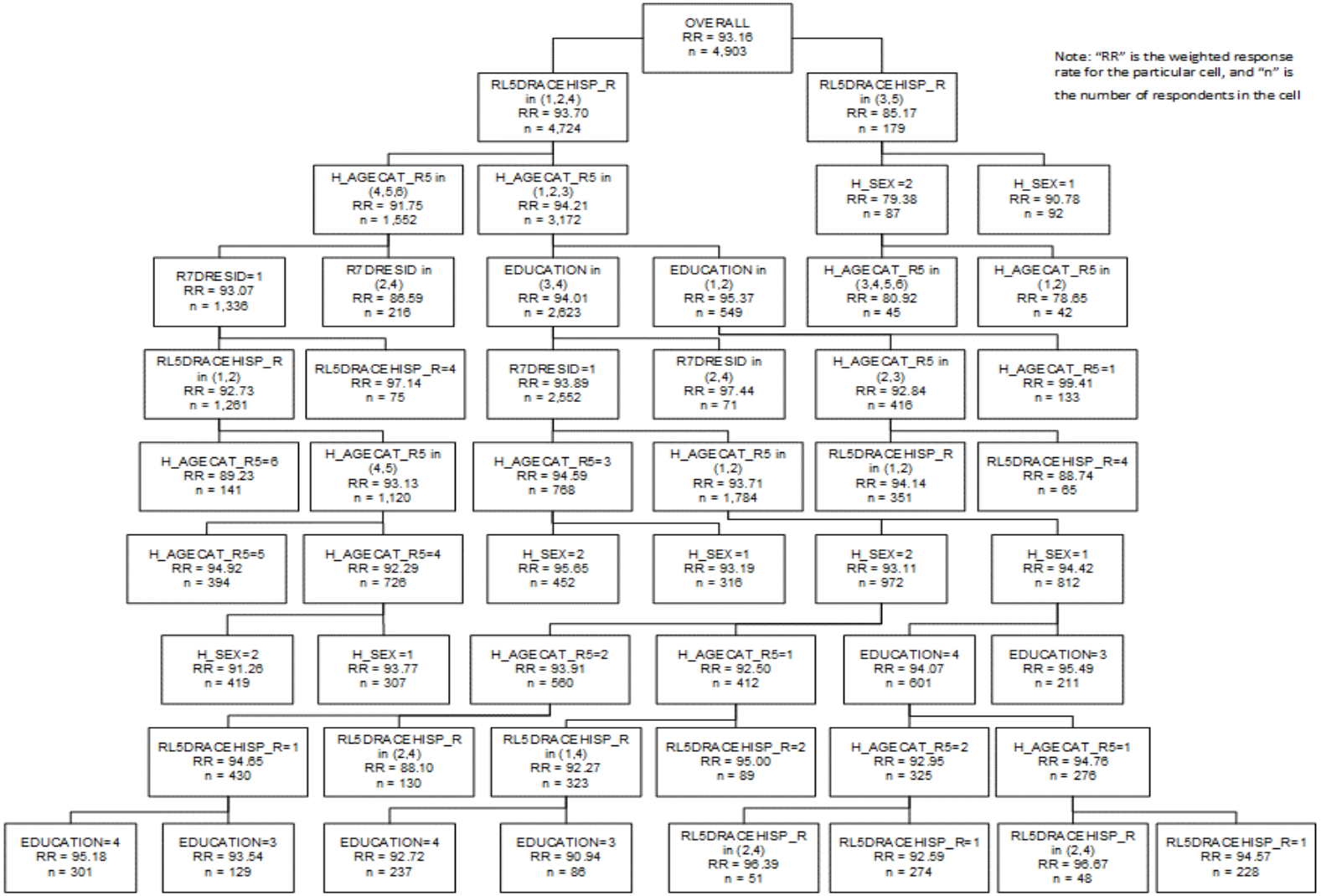


Figure 3. Round 7 2015 Cohort DBS Weight Nonresponse Adjustment Cells – adjusting for no valid assay results