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Add Health Wave IV Documentation

Measures of Inflammation and Immune Function

Report prepared by

Eric A. Whitsel
Carmen C. Cuthbertson
Joyce W. Tabor
Alan J. Potter
Mark H. Wener
Ley A. Killeya-Jones
Kathleen Mullan Harris

Carolina Population Center
University of North Carolina at Chapel Hill
123 Franklin Street, Room 403-B
Chapel Hill, NC 27516-2524

Add Health

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1. Introduction

During Wave IV, Add Health collected biological specimens from a large, nationally representative sample of young adults. Given the size of the Wave IV sample, its geographic distribution, and in-home setting of the respondent interviews, biological specimen collection involved practical, relatively non-invasive, cost-efficient and innovative methods. These methods included collection of capillary whole blood via finger prick by trained and certified field interviewers, its *in situ* desiccation, then shipment, assay and archival of dried blood spots. The collection of capillary whole blood followed the collection of cardiovascular and anthropometric measures (Entzel et al, 2009) and saliva (Smolen et al, 2012). It preceded the collection of data on respondent use of prescription and select over-the-counter medications (Tabor et al, 2010). Further details on the design of Add Health Waves I-IV, are available elsewhere (Harris, 2011).

Included in the Add Health Wave IV data are two measures of inflammation and immune function based on assay of the dried blood spots:

- High Sensitivity C-Reactive Protein (hsCRP, mg/L) and
- Epstein Barr Viral Capsid Antigen IgG (EBV, AU/ml)

To facilitate analysis and interpretation of hsCRP and EBV, the restricted-use Add Health Wave IV data also include two data quality flags and 11constructed measures:

- CRP_FLAG
- EBV_FLAG
- Classification of hsCRP (Pearson et al, 2003)
- Count of Common Subclinical Symptoms (Vaidya et al, 2006)
- Count of Common Infectious or Inflammatory Diseases
- NSAID/Salicylate Medication Use in the Past 24 Hours
- NSAID/Salicylate Medication Use in the Past 4 Weeks
- Cox-2 Inhibitor Medication Use in the Past 4 Weeks
- Inhaled Corticosteroid Medication Use in the Past 4 Weeks
- Corticotropin/Glucocorticoid Medication Use in the Past 4 Weeks
- Anti-rheumatic/Anti-psoriatic Medication Use in the Past 4 Weeks
- Immunosuppressive Medication Use in the Past 4 Weeks
- Anti-inflammatory Medication Use

This document summarizes the rationale, equipment, protocol, assay, internal quality control, data cleaning, external quality control, and classification procedures for each measure listed above. Measures of glucose homeostasis and candidate genes are documented elsewhere

(Whitsel et al, 2012; Smolen et al, 2012). Documentation of lipids will be provided in a separate report.

2. General Overview of Data Collection

A Blaise computer-assisted interview (CAI) program guided trained and certified field interviewers (FIs) through the blood spot collection process. Help screens with step-by-step measurement instructions were accessible within the program. Each FI also carried a Job Aids Booklet that served as a quick reference guide to study protocols.

Respondents were free to decline any or all measurements and specimen collections while participating in other components of the interview. In the Wave IV data set, any measures that are missing due to unique circumstances at correctional facilities are coded as legitimate skips.

Some measurement protocols were revised in the period between the Wave IV Pretest (conducted in 2007) and the Main Study (conducted in 2008). Where the Pretest and Main Study data collection protocols differed significantly, this report documents the key differences between them. Pretest cases in the Wave IV data set are flagged for identification.

3. Capillary Whole Blood Collection

3.1 Rationale

Capillary whole blood was collected to provide Add Health with the biological specimens necessary to assay and interpret a pre-specified panel of metabolic, inflammatory, and immune biomarkers, including the measures of high sensitivity C-reactive protein (hsCRP) and Epstein-Barr viral capsid antigen IgG (EBV) described herein. It also was collected to establish a dried capillary whole blood spot archive capable of supporting future assays and ancillary studies.

Exhibit 1. Capillary whole blood collection equipment
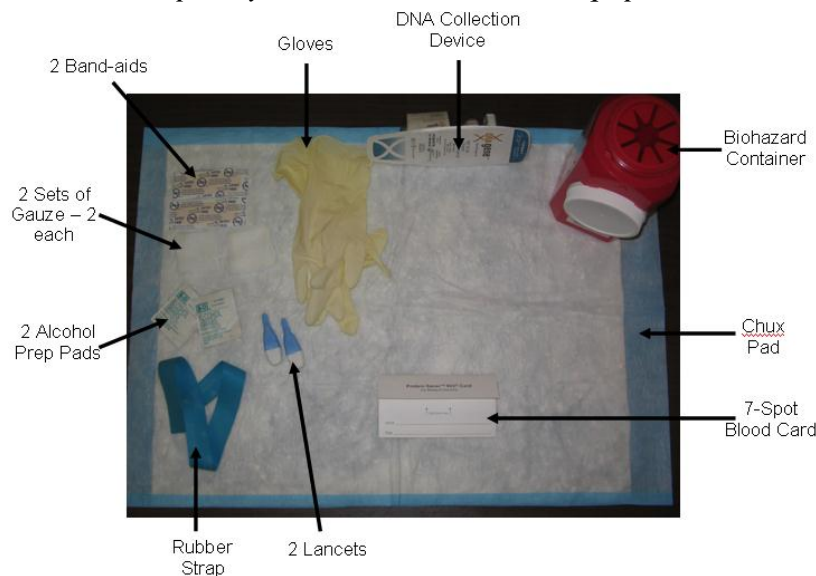
3.2 Equipment

Equipment included sterile lancets, rubber strap, alcohol prep pads, gauze, Band-aid type adhesive dressings, gloves, biohazard container, Chux-type absorbent underpad, and a seven-spot capillary whole blood collection card (Whatman 903® *Protein Saver*, Whatman Inc., Piscataway, NJ) to which a stabilizing, buffered preservative had been pre-applied to Spot #1 (*Exhibit 1*).

3.3 Protocol

3.3.1 Main Study

During the preceding anthropometric data collection procedure, all female respondents were asked specifically whether they had a prior mastectomy and, if so, on which side. If there were contraindications to using the right hand for capillary whole blood collection, the left hand was used. If there were contraindications on both hands, capillary whole blood was not collected.

FIs collected capillary whole blood from the respondent's middle or ring finger, unless one of the following contraindications was present:

- open sores, wounds, gauze dressings or rashes;
- casts, splints or shunts;
- intravenous (IV) catheters or other attached medical devices;
- swelling, withering or paralysis; or
- finger on same side as prior mastectomy.

FIs prepared the work surface for capillary whole blood collection and donned gloves. The help screen on the computer laptop directed FIs to refer to the Job Aids Booklet for the Fainting Protocol. FIs selected a finger for the procedure, cleaned it with the alcohol prep pad, and let it fully dry. While the finger was drying, FIs asked respondents to hang the selected finger below their waist while applying the rubber strap to the midpoint of the upper ipsilateral arm. After placing the rubber strap, respondents started a timer on the laptop computer designed to sound an audible cue after three minutes to prompt removal of the rubber strap. FIs placed the clean finger against the work surface and firmly placed a sterile lancet against it to prick the fingertip, slightly lateral of center. FIs firmly wiped away the first drop of capillary whole blood with gauze, applying pressure to the base of (but not milking) the finger to facilitate flow. FIs were trained to allow a large droplet to accumulate before dropping it onto the first circle of the seven-spot capillary whole blood collection card and to do the same for the remaining six circles from left to right, all without allowing the fingertip to touch the card (*Exhibit 2*).

Exhibit 2. Collecting the capillary whole blood.

When seven capillary whole blood spots were successfully collected (or blood droplet formation ceased), FIs wiped off remaining blood with gauze, instructed respondents to firmly apply the gauze to the finger for at least two minutes, and then applied a band aid to it. FIs collecting fewer than five spots less than 80% full from a single prick requested respondents' permission to repeat the capillary whole blood collection procedure on a second finger from the contralateral hand. FIs asked respondents to discard used capillary whole blood collection equipment in their own trash receptacle (except for lancets which were discarded in the biohazard container). FIs discarded them in the biohazard container when interviews were conducted in public locations.

FIs bar code labeled each capillary whole blood spot collection card with the corresponding respondent biospecimen ID and then air dried it for three hours. Thereafter, FIs packaged each card with a desiccant pack and shipped it in a FedEx Priority Overnight envelope to the University of Washington Department of Laboratory Medicine (UW Lab Med, Mark H. Wener, M.D., Director, Seattle, WA) for assay.

UW Lab Med received the FedEx Priority Overnight envelopes containing a single dried blood spot collection card and desiccant pack. They scanned the FedEx tracking number and bar code-labeled card into a database in the order of receipt. They also keyed the receipt date, number of dried blood spots per card (0-7), number of adequate blood spots per card defined by blood filling $\geq$ 80% of the target area (0-7), comments on dried blood spot quality, and condition of the desiccant pack alongside the biospecimen ID. They grouped the cards ($\leq$ 25 per group), sealed the groups in Ziploc bags with desiccant packs, and stored them at -70°C until processing. Immediately before processing, they warmed cards to room temperature (23°C) and re-scanned the bar code-labeled card into the database. The cards were punched for all assays except hemoglobin A_{1c} (HbA$_{1c}$), returned to the freezers, then shipped frozen to the Carolina Population Center (CPC, University of North Carolina, Chapel Hill, NC) for permanent archival. At the CPC, Spot #1 on each card was removed, re-bundled ($\leq$ 25 per group), sealed in plastic bags with desiccant, and shipped frozen by next day air to FlexSite Diagnostics, Inc. (Robert A. Ray, Ph.D., Director, Palm City, FL) for HbA$_{1c}$ assay.

3.3.2 Pretest Methodological Variations

During the Pretest, respondents chose the middle or ring finger and FIs were directed not to use the thumb, index finger or fifth digit/little finger for capillary whole blood collection. FIs also

collected up to ten capillary whole blood spots: three on a BIOSAFE Blood Collection Card for HbA$_{1c}$ and Cholesterol Panel (BIOSAFE Laboratories, Inc., Chicago, IL) and seven more on a Whatman 903® Protein Saver, (Whatman Inc., Piscataway, NJ). The BIOSAFE card was made of Whatman 903® filter paper (Whatman International, Dassel, Germany) with a top layer of TELFA (Kendall Healthcare Products, Mansfield, MA) to minimize the effects of blood spot layering and inadvertent touching of cards with respondent fingertips (Tyrrell, 1999; Maggiore, 2002; Bui et al, 2002a; Bui et al, 2002b; Grzeda et al, 2002). A stabilizing borate buffered preservative was pre-applied by BIOSAFE to the area of the BIOSAFE card designated for HbA$_{1c}$ assay and dried. As in the main study, the Whatman 903® Protein Saver card was shipped to UW Lab Med for assay.

4. Measures of Inflammation and Immune Function

4.1 High Sensitivity C-Reactive Protein (hsCRP)

4.1.1 Rationale

CRP is produced by the liver in response to inflammation. It also is a fairly stable protein that can be sensitively measured with precision using standardized laboratory procedures (Pearson et al, 2003). Moreover, in asymptomatic, intermediate-risk men aged ≤ 50 years and women ≤ 60 years, measurement of hsCRP may be useful in cardiovascular risk assessment (Greenland et al, 2010). It was therefore assayed in dried capillary whole blood spots.

4.1.2 Assay and Internal Quality Control

UW Lab Med constructed dried blood spot CRP assay calibrators from pooled human plasma with a negligible CRP concentration (negligible CRP plasma; UW Lab Med) spiked with CRP concentrate (Cell Sciences, Canton, MA) and serially diluted with negligible CRP plasma to the desired final CRP concentration. Four dried blood spot quality control samples were constructed from a separate pool of human plasma, either undiluted (high CRP concentration quality control sample) or diluted with negligible CRP plasma to the desired final CRP concentrations: medium-high, medium, and low. Each calibrator and quality control sample solution was mixed with a constant volume of washed human erythrocytes (UW Lab Med), pipetted in 75μl aliquots onto #903 filter paper (Whatman, Piscataway, NJ) and dried for 2 hours at room temperature (23°C). The CRP concentration of each calibrator and quality control sample was determined by analysis of plasma from the sample solution-erythrocyte mixture on a UniCel DxC 800 Synchron Clinical System (Beckman Coulter, Miami, FL) or a Siemens Dade Behring BN II Nephelometer (Charlotte, NC). Dried blood spot calibrators and quality control samples were sealed at -70ºC in Ziploc bags with desiccant until use.

A single 3.2 mm (1/8 in) diameter punch was taken from each dried blood spot calibrator, quality control sample, or respondent sample card and placed into a deep-well microtiter plate well (Greiner Bio-One, Monroe, NC). Plates were either immediately assayed or were firmly sealed and stored at -70°C pending assay.

The sandwich ELISA used to measure CRP in Add Health was adapted from a previously published method (McDade et al, 2004). Details of the enzyme-linked immunosorbent assay are provided below, but in brief, punches from dried blood spot calibrator, quality control sample, or respondent sample cards were eluted in a buffer solution and the eluent transferred to wells on an ELISA microtiter plate, the bottom faces of which were pre-coated with a CRP-recognizing monoclonal antibody (mAb). CRP in the eluent was bound by the anti-CRP mAb (solid phase immobilization). A conjugate solution containing an anti-CRP Ab coupled to peroxidase (enzyme-linked antibody) was then added to each well, sandwiching the CRP molecules between the solid phase and enzyme-linked antibodies. After incubation, the wells were washed to remove unbound material. A tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) solution was added. H_2O_2, cleaved by the peroxidase, reacted with TMB and caused the solution to develop color. The absorbance (optical density) of the calibrators was spectrophotometrically measured and plotted against their known CRP concentrations. Using the calibration curve, the optical densities of the quality control and respondent samples were read as CRP concentrations.

Plates to be assayed were warmed to room temperature and then 111 µl CRP elution buffer (hsCRP Sample Diluent; Percipio, Inc, Manhattan Beach, CA) was added to each microtiter plate well. The plate was sealed and gently shaken for 1 hour on a Delfia Plateshake microplate shaker (PerkinElmer, Waltham, MA) to elute CRP. A 10 µl aliquot of eluent was transferred in duplicate from each well of the elution plate to an ELISA microtiter plate (Percipio). Also added in duplicate to ELISA microtiter plate wells were 10 µl aliquots from two quality control samples: one each of normal and low CRP concentration serum (UW Lab Med) diluted 1:100 with PBS/BSA (Sigma, St. Louis, MO). 100 µl of CRP Enzyme Conjugate Reagent (Percipio) was added to each well, the plate was gently shaken for 30 seconds, incubated at room temperature for 45 minutes in the dark, and then washed five times with di/ddH$_2$O. 100µl of TMB Reagent (Percipio) was added, the plate gently shaken for 30 seconds and then placed on a microtiter plate reader (Synergy HT, BioTek, Winooski, VT). The optical density of each well was read at 370 nm after the optical density of the high calibrator on the plate reached 1.5 (approximately 20 minutes). A calibration curve was constructed by plotting the assigned calibrator concentrations against the recorded optical densities. The calibration curve was used to convert the optical density of each sample into a dried blood spot CRP concentration (Gen 5 Software, BioTek). Acceptability of the assay was determined by comparing the CRP concentrations of the quality control samples with their established values. The sensitivity of the CRP assay was 0.035 mg/L (plasma equivalent of 0.082), the within-assay coefficient of variation was 8.1%, and between-assay coefficient of variation was 11.0%. CRP concentrations (mg/L) of 87 dried blood spot and paired plasma samples were strongly correlated and linearly

associated: Pearson R = 0.98; Plasma CRP = DBS CRP / 0.4285. Due to the depletion of the original standards, one DBS sample was converted using a different set of standards: Pearson R = 0.99; Plasma CRP = DBS CRP / 01.282.

4.1.3 Pretest Methodological Variations

None.

4.2 Epstein-Barr Viral Capsid Antigen IgG (EBV)

4.2.1 Rationale

EBV was assayed in dried capillary whole blood spots because there is persuasive evidence that EBV is among the strongest and most consistent immunological correlates of chronic stress.

4.2.2 Assay and Internal Quality Control

Dried blood spot EBV assay calibrators and quality control samples were supplied by the ELISA manufacturer (DiaSorin, Stillwater, MN). UW Lab Med constructed four dried blood spot quality control samples from separate pools of human serum: negative (DiaSorin), low, medium or high concentrations of EBV (UW Lab Med). Quality control sample serum was mixed with a constant volume of washed human erythrocytes (UW Lab Med), pipetted in 75 µl aliquots onto #903 filter paper (Whatman, Piscataway, NJ) and dried for 2 hours at room temperature (23°C). The final EBV concentration of each dried blood spot quality control sample was determined from repeated analysis of the sample on an EBV ELISA (Diasorin). Dried blood spot calibrators and quality control samples were stored at -70°C in sealed Ziploc bags with desiccant until use.

A single 3.2 mm (1/8 in) diameter punch was taken from each dried blood spot calibrator, quality control sample, or respondent sample card and placed into a deep-well microtiter plate well (Greiner Bio-One, Monroe, NC). Plates were either immediately assayed or were firmly sealed and stored at -70°c pending assay.

The indirect ELISA used to measure the concentration of EBV in Add Health was adapted from a previously published method (McDade et al, 2000). Details of the indirect ELISA are provided below, but in brief, punches from dried blood spot calibrator, quality control sample, or respondent sample cards were eluted in a buffer solution and the eluent transferred to wells on an ELISA microtiter plate, the bottom faces of which were pre-coated with the Epstein-Barr viral capsid p18 peptide recognized by the EBV viral capsid antigen IgG. EBV viral capsid antigen IgG in the transferred solutions complexed with the bound p18 peptide (solid phase immobilization). A conjugate solution containing an anti-IgG antibody coupled to peroxidase (enzyme-linked antibody) was then added to each well, simultaneously binding the EBV viral

capsid antigen IgG by the immobilized solid phase and the enzyme-linked antibodies. After incubation, the wells were washed to remove unbound material. A tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) solution was added. H_2O_2, cleaved by the peroxidase, reacted with TMB and caused the solution to develop color. The reaction was stopped after a fixed duration. The absorbance (optical density) of the calibrators was measured spectrophotometrically and plotted against their known EBV concentrations. Using the calibration curve, the optical densities of the quality control and respondent samples were read as EBV concentrations.

Plates to be assayed were warmed to room temperature and then 111µl CRP elution buffer (hsCRP Sample Diluent; Percipio, Inc, Manhattan Beach, CA) was added to each microtiter plate well. The plate was sealed and gently shaken for 1 hour on a Delfia Plateshake microplate shaker (PerkinElmer, Waltham, MA) to elute EBV. A 20 µl aliquot of eluent was extracted for the dried blood spot CRP assay and the remaining 91 µl was then mixed with 270 µl Sample Diluent (DiaSorin). 100 µl was transferred from each well of the elution plate, and from each calibrator and quality control sample solution, to an ELISA microtiter plate (DiaSorin). All calibrators, controls and samples were assayed in duplicate. The plate was gently shaken for 30 seconds, incubated at 37° for 60 minutes in the dark, and then washed five times with Wash Buffer (DiaSorin). 100 µl of Diluted Enzyme Tracer (DiaSorin) was added to each ELISA microtiter plate well, the plate incubated at 37° for 60 minutes in the dark, and then washed five times with Wash Buffer (DiaSorin). 100 µl of Chromagen/Substrate (DiaSorin) was added, the plate gently shaken for 30 seconds, incubated at room temperature for 15 minutes in the dark, and then 100 µl of Stop Solution (DiaSorin) was added. The plate was placed on a microtiter plate reader (Synergy HT, BioTek, Winooski, VT) and the optical density was read at 450 nm. A calibration curve was constructed by plotting the assigned calibrator concentrations against the recorded optical densities. The calibration curve was used to convert the optical density of each sample into a dried blood spot EBV concentration (Gen 5 Software, BioTek). Acceptability of the assay was determined by comparing the EBV concentrations of the quality control samples with their established values. The sensitivity of the EBV assay was 9 AU/ml (plasma equivalent of 25), the within-assay coefficient of variation was 3.9%, and between-assay coefficient of variation was 10.2%. EBV concentrations (AU/ml) of 162 dried blood spot and paired serum samples were strongly correlated and linearly associated: Pearson r = 0.95; Plasma EBV = (DBS EBV + 4.579) / 0.575.

4.2.3 Pretest Methodological Variations

None.

5. Data Cleaning and External Quality Control

5.1 Data Cleaning

Dried blood spot respondent samples yielding high between-duplicate differences were flagged (*Section 6.1*) and when possible, re-assayed in duplicate. The assayed (or in their place, re-assayed) duplicate values were then averaged. Plate-specific, linear regression calibration formulae (*Sections 4.1.2 and 4.2.2*) were used to verify laboratory conversion of optical density to EBV (AU/ml). Dried blood spot respondent samples yielding concentrations in the lowest half percentile of averaged duplicates were also re-assayed in duplicate, when possible. In this case, the original and re-assayed values were averaged. In addition, average values exceeding the upper range of calibration curves were flagged. Seasonal variation was examined by plotting and modeling participant sample concentrations versus assay date (2007-2010) on a single calendar time scale (1-366 days), but there was little graphical or statistical evidence of it (*Exhibit 4*).

Exhibit 4. Mean hsCRP (mg/L) and EBV (AU/ml) versus assay day

△ High control ■ Participants ▽ Low control
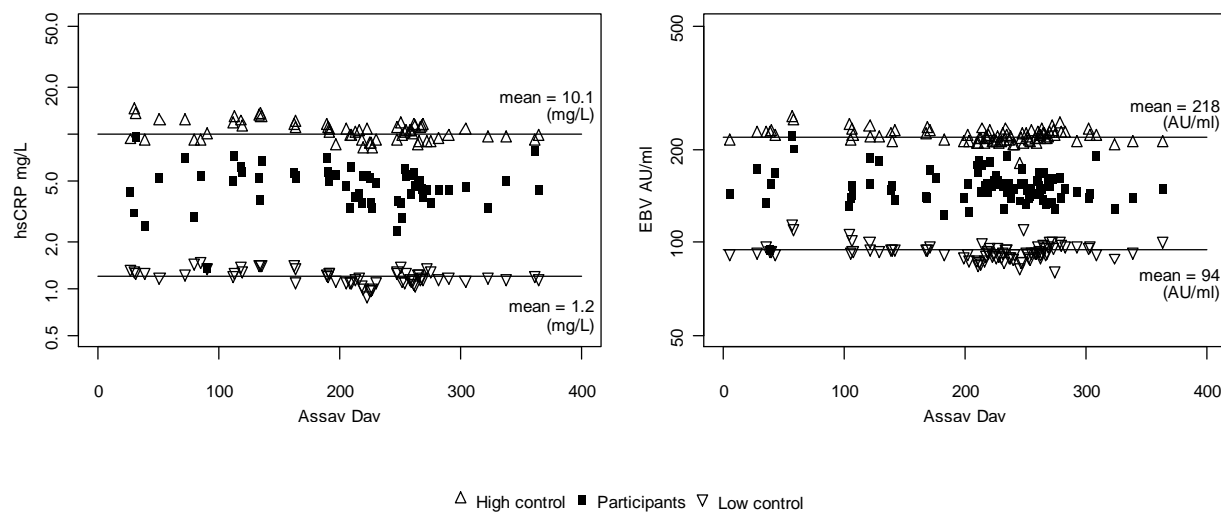
5.2 External Quality Control

Within a race / ethnicity- and sex-stratified random sample of (n = 100) Add Health respondents among whom capillary whole blood was collected twice, one-two weeks apart, reliabilities of hsCRP (mg/L) and EBV (AU/ml) were estimated as intra-class correlation coefficients (95% CI): 0.70 (0.59-0.81) and 0.97 (0.96-0.98), respectively.

6. Constructed Measures

6.1 Flags

Variables: CRP_FLAG and EBV_FLAG
Flags were constructed to identify between-duplicate differences greater than three standard deviations and average values exceeding the upper ranges of calibration curves (*Section 5.1*).

6.2 Classification of hsCRP

Variable: C_CRP
The classification of hsCRP concentrations among Add Health respondents was constructed without regard to fasting status, in accordance with the American Heart Association / Centers for Disease Control clinical and public health practice recommendations regarding markers of inflammation and cardiovascular disease (Pearson et al, 2003). In keeping with the recommendations, classes of hsCRP were defined to approximate tertiles in the adult population, as tabulated below, although it should be noted that in many populations, only 5% of hsCRP concentrations exceed 10 mg/L:

hsCRP (mg/L)	AHA/CDC Class
< 1	Low
1-3	Average
> 3	High

6.3 Count of Common Subclinical Symptoms

Variable: C_SUBCLN
High hsCRP concentrations, particularly those exceeding 10 mg/L, should trigger searches for non-cardiovascular (e.g. infectious or inflammatory) diseases capable of seriously confounding hsCRP-based estimates of cardiovascular disease risk. Subclinical sources of infection or inflammation identified in *Section 6: Illness, Medications and Physical Disabilities* of the Wave IV in-home interview also have potential to confound hsCRP-based estimates of cardiovascular disease risk in apparently healthy populations. Common symptoms identified by items H4ID10A - H4ID10G were therefore counted and categorized as previously described (Vaidya et al, 2006; Becker, 2012; CDC, 2012) for investigation or control of potential confounding in hsCRP analyses:

Symptom Count	Category
0	0
1	1
2	2
≥ 3	3+

6.4 Count of Common Infectious or Inflammatory Diseases

Variable: C_INFECT
Although the infectious and inflammatory diseases identified in *Section 6: Illness, Medications and Physical Disabilities* are not included in the symptom count tabulated above, they too may

confound hsCRP-based estimates of cardiovascular disease risk. Therefore, responses to items H4ID5F, H4ID5N, and H4ID9A - H4ID9F from this section were counted and categorized for investigation or control of potential confounding in hsCRP analyses:

Symptom Count	Category
0	0
1	1
2	2
≥ 3	3+

6.5 Medication Use Variables

Variables: CRP_MED1 – CRP_MED8

Use of anti-inflammatory medications (and / or the diseases for which they are being taken) also may confound hsCRP-based estimates of cardiovascular disease risk. These exposures were captured at Wave IV in the medication inventory (Tabor et al, 2010), and for salicylates / nonsteroidal anti-inflammatory drugs (NSAIDs), in *Section 6: Illness, Medications and Physical Disabilities*. They should be used cautiously in the investigation or control of potential confounding in hsCRP analyses because the typical intermittency and brevity of anti-inflammatory medication use (e.g. for headache, menstrual cramps, muscle ache, etc.) and their short half-lives in the circulation reduce ability to accurately define exposure. Moreover, selection biases often threaten the study of non-randomized medication exposures.

Table. Eight variables capturing use of anti-inflammatory medications

Variable	Label	Variables Used in Construction
CRP_MED1	NSAID/Salicylate Medication Use in the Past 24 Hours	H4ID11=yes or H4ID12=yes
CRP_MED2	NSAID/Salicylate Medication Use in the Past 4 Weeks	057-058-061 Nonsteroidal Anti-Inflammatory Agents or 057-058-062 Salicylates or Any other oral medication containing an NSAID or salicylate*
CRP_MED3	Cox-2 Inhibitor Medication Use in the Past 4 Weeks	057-058-278 Cox-2 Inhibitors
CRP_MED4	Inhaled Corticosteroid Medication Use in the Past 4 Weeks	122-130-296 Inhaled Corticosteroids
CRP_MED5	Corticotropin/Glucocorticoid Medication Use in the Past 4 Weeks	097-098-300 Corticotropins or 097-098-301 Glucocorticoids
CRP_MED6	Antirheumatic/Antipsoriatic Medication Use in the Past 4 Weeks	105-192-*** Antirheumatics or 105-270-*** Antipsoriatics
CRP_MED7	Immunosupressive Medication Use in the Past 4 Weeks	254-104-*** Immunosuppressive agents or 254-257-*** Immunosuppressive monoclonal antibodies
CRP_MED8	Anti-inflammatory Medication Use	Reported any of the above medications

*As an ingredient in a combination medication.

7. References

Becker DM. *The Johns Hopkins Sibling and Family Heart Study / Genetic Study of Atherosclerosis Risk (Gene STAR)*, 2012. Available at http://www.genestarstudy.com/Background-and-History.html.

Bui TL, Warner CN, Ildefonso J, Pirucki TL, Dewey LM, Maggiore JA. Development of a dried blood collection, transport and testing system for HDL-Cholesterol. *Clin Chem* 2002; 48(S): A97.

Bui TL, Warner CN, Ildefonso J, Pirucki TL, Dewey LM, Maggiore JA. Development of a dried blood collection, transport and testing system for total cholesterol. *Clin Chem* 2002; 48(S): A98.

Centers for Disease Control and Prevention (CDC). National Health Interview Survey (NHIS), 2012. Available at http://www.cdc.gov/nchs/nhis.htm.

Entzel P, Whitsel EA, Richardson A, Tabor J, Hallquist S, Hussey J, Halpern CT, Harris KM. *Add Health Wave IV documentation: Cardiovascular and anthropometric measures*. UNC Chapel Hill: Carolina Population Center, 2009. Available at http://www.cpc.unc.edu/projects/addhealth/data/guides/Wave%20IV%20cardiovascular%20and%20anthropometric%20documentation%20110209.pdf.

Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, Foster E, Hlatky MA, Hodgson JM, Kushner FG, Lauer MS, Shaw LJ, Smith SC Jr, Taylor AJ, Weintraub WS, Wenger NK, Jacobs AK; American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation* 2010; 122(25):e584-636.

Grzeda BR, Bui TL, Warner CN, Pirucki TL, Dewey LM, Maggiore JA. Development of a dried blood collection, transport and testing system for triglycerides. *Clin Chem* 2002; 48(S):A106.

Harris KM. *Design features of Add Health*. UNC Chapel Hill: Carolina Population Center, 2011. Available at http://www.cpc.unc.edu/projects/addhealth/data/guides/design%20paper%20WI-IV.pdf

Maggiore JA. *BIOSAFE Safe At Home Cholesterol Profile Blood Collection and Transport System.* US Food and Drug Administration, 510(k) Over-the-Counter Approval, 4/3/02: K012221, 2002.

McDade TW, Burhop J, Dohnal J. High sensitivity enzyme immunoassay for C reactive protein in dried blood spots. *Clin Chem* 2004; 50:652-654.

McDade TW, Stallings JF, Angold A, Costello EJ, Burleson M, Cacioppo JT, Glaser R, Worthman CM. Epstein-Barr virus antibodies in whole blood spots: A minimally invasive method for assessing an aspect of cell-mediated immunity. *Psychosom Med* 2000; 62(4): 560-568.

Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC Jr, Taubert K, Tracy RP, Vinicor F, Centers for Disease Control and Prevention; American Heart Association. Markers of inflammation and cardiovascular disease. Application to clinical and public health practice. A statement for healthcare professionals from the CDC and AHA. *Circulation* 2003; 107(3):499-511.

Smolen A, Whitsel EA, Tabor J, Killeya-Jones LA, Cuthbertson CC, Hussey J, Halpern CT, Harris KM. *Add Health Wave IV Documentation: Candidate Genes*. UNC Chapel Hill: Carolina Population Center, 2012. Available at http://www.cpc.unc.edu/projects/addhealth/data/guides/DNA_documentation.pdf

Tabor J, Whitsel EA. *Add Health Wave IV Documentation: Prescription Medication Use*. UNC Chapel Hill: Carolina Population Center, 2010. Available at http://www.cpc.unc.edu/projects/addhealth/data/guides/medication_documentation.pdf.

Tyrrell SP. *BIOSAFE Safe At Home Test Kit for Monitoring Hemoglobin A1c.* US Food and Drug Administration, 510(k) Over-the-Counter Approval; 11/26/99: K991850, 1999.

Vaidya D, Yanek LR, Moy TF, Faraday N, Hasan AA, Becker LC, Becker DM. Common subclinical symptoms in apparently healthy people without active infections are associated with high-sensitivity C-reactive protein. *Circulation* 2006; 113(8):E336.

Whitsel EA, Nguyen QC, Suchindran CM, Tabor JW, Cuthbertson CC, Wener MH, Potter AJ, Killeya-Jones L, Hussey JM, Halpern CT, Harris KM. *Dried capillary whole blood spot-based hemoglobin A1c, fasting glucose, and diabetes prevalence in a nationally representative population of young U.S. adults: Add Health, Wave IV.* Am Heart Assoc CVD Epid Prev / Nutr Phys Act Metab Sci Sessions, 2012.