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Add Health Wave V Documentation



Renal Function

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Add Health is supported by grant P01-HD31921 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development, with cooperative funding from 23 other federal agencies and foundations.

This document summarizes the rationale, equipment, protocol, assay, internal quality control, data cleaning, external quality control, and procedures for the measurement and classification of kidney (renal) function at the Wave V home exam. Whenever possible, data collection and methods in Wave V mirrored those of Wave IV to ensure comparability of data between waves, although **important inter-Wave differences exist and are grey highlighted herein**. This document is one in a set of Wave V user guides. User guides are also available to describe protocols for the following biological measures at Wave V:

- Anthropometrics
- Cardiovascular Measures
- Medication Use – Home Exam
- Baroreflex Sensitivity, & Hemodynamic Recovery
- Glucose Homeostasis
- Inflammation and Immune Function
- Lipids

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1. Introduction

Renal function was not measured at Wave IV, but was measured at Wave V. Briefly, blood was collected by field examiners (FEs) certified in phlebotomy, chilled at 4°C during the remainder of the home exam, centrifuged immediately afterward, aliquoted into transport tubes, and then sent overnight to a laboratory for assay.

Assayed Renal Biomarkers:

- Creatinine (mg/dL)
- Cystatin C (mg/L)

Moreover, the restricted use Add Health Wave V data includes nine constructed measures designed to facilitate analysis and interpretation of renal function:

Estimated Glomerular Filtration Rate (eGFR, ml/min/1.73 m²) According to NIDDK CKD-EPI Guidelines.¹

- Based on creatinine
- Based on cystatin C
- Based on both creatinine and cystatin C

Classifications of eGFR According to KDIGO Guidelines²

- Based on creatinine
- Based on cystatin C
- Based on both creatinine and cystatin C

Clinical Classifications of eGFR

- Based on creatinine
- Based on cystatin C
- Based on both creatinine and cystatin C

2. General Overview of Data Collection

All Wave V venous blood samples were collected during home exams performed by FEs from two Add Health data collection partners: Examination Management Services, Inc. (2016–2017) and Hooper Holmes, Inc. (2018–2019). All FEs were trained and certified using a custom program specific to the Add Health protocol. FEs used a 7" Samsung Galaxy Tab 4 tablet to record and transmit data. An Add Health data collection application (Open Data Kit or ODK) installed on the tablet guided the FEs through the home exam protocol. In addition, FEs received a series of job aids, both on paper and on the tablet, to serve as quick reference guides when completing the protocol. Each tablet also contained an in-depth Add Health training manual that could be accessed at any time.

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FEs conducted home exams among previously consented respondents. All FEs were phlebotomy-certified and had at least two years of experience collecting venous blood. Before home exams, FEs were sent a Visit Supply Kit that included a box for shipping blood to the lab and a Blood Collection Kit containing most required materials for the blood collection. FEs supplied additional materials, as needed (see section 3.2). Protocols for blood collection were dictated to FEs by the handheld 7" Samsung tablet used during all home exams. The tablet gave step-by-step directions for the blood collection and required FEs to enter information about the blood draw for each respondent. All respondents had the option to decline part or all of the blood draw, although declining did not affect their ability to participate in the rest of the home exam. Overall, 91.8% of the respondents agreed to and completed the blood draw. Of the remainder, 6.5% refused, 1.3% agreed but the blood draw was unsuccessful, and < 1% had exams terminated before the blood draw (see the blood draw status variable **H5BLOOD** in the *bdemo5* data set and codebook).

Blood collection was the last step in the home exam. Afterwards, all collection tubes were inverted 8-10 times to distribute the blood and contents of the tubes and then chilled at 4° C (on ice or frozen cold packs) for up to two hours. Subsequent processing involved centrifuging specific tubes then aliquoting serum and plasma into color-coded transport tubes pre-labelled with unique barcode identifiers linking the blood to a particular respondent. Then the transport tubes were packaged in a Styrofoam Box with frozen cold packs and shipped overnight via FedEx to the Laboratory for Clinical Biochemistry Research (LCBR) at the University of Vermont. Overnight shipment enabled receipt by LCBR before 10:30 am the next morning. Upon receipt, LCBR documented the arrival of the transport tubes, evaluated their condition, processed them, and either assayed the specimens or aliquoted and archived them in -80°C freezers.

3. Blood Collection

3.1 Rationale

Venous blood was collected to provide Add Health with the biological specimens necessary to assay and interpret a pre-specified panel of metabolic, hematologic, inflammatory, immune, and renal biomarkers, including the measures of renal function described herein. It also was collected to establish an archive of serum, plasma, whole blood, RNA, and packed cells capable of supporting future assays and ancillary studies.

3.2 Equipment

Before exams, FEs were shipped a Visit Supply Kit (Exhibit 1) including (1) a cardboard Shipping Box with an inner Styrofoam Box and two cold packs for shipping collected samples to LCBR, (2) a large Tyvek envelope in which to ship the Shipping Box, and (3) a Blood Collection Kit for collecting blood. The Blood Collection Kit contained:

- Biohazard-labelled Ziploc bag
- Latex-free gloves
- 2"x2" gauze

- Latex-free, Band-Aid type adhesive dressings
- Latex-free, strap tourniquet
- Alcohol prep pads, disposable pipets
- Single-use vacutainer (blood collection) tube holder
- 21-gauge Eclipse straight needle
- 21-gauge butterfly needle
- (3) disposable 3 ml graduated transfer pipets
- (2) 8.5 ml serum separation transport (SST) vacutainer tubes
- (1) 6 ml sodium fluoride/potassium oxalate (NaF/KOx)-containing vacutainer tube, if needed for the glucose sub-study (see Section 4.1.2.1)
- (1) 3- or 4- ml potassium ethylenediaminetetraacetic acid (EDTA)-containing vacutainer tube
- (1) 10 ml EDTA-containing vacutainer tube
- (1) 10 ml PAXgene vacutainer tube (containing 7.5 ml of preservative)
- (4) 10 ml transport tubes with color coded caps
- Extra barcode labels

BD Biosciences (San Jose, CA) supplied all the vacutainer and transport tubes. As of February 2018, their 3 ml EDTA vacutainer tube (Cat #367835) was no longer available, so Add Health switched to the 4 ml EDTA vacutainer tube (Cat #367844).



Exhibit 1. Visit Supply and Blood Collection Kits

FEs were responsible for providing ancillary materials for each home exam, including but not limited to a chux-type absorbent under pad, a sharps container, and a cooler with cold packs for keeping samples cold before packaging and shipping them to LCBR.

3.3 General Protocol

3.3.1 Blood Collection

The blood draw was performed as the final stage of the home exam following collection of anthropometric, cardiovascular, and medication information. After confirming respondents were comfortable giving blood, respondents were asked to either sit or recline at their discretion. They also

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were asked if they had problems in the past with blood collection such as fainting, bleeding, or hard-to-find veins. FEs were instructed to ensure the blood collection area was private, uncluttered, and fully prepared before beginning the blood draw. Preparation involved placing the chux pad, organizing the vacutainer tubes/supplies, preparing the cooler to accept the blood samples, and scanning the barcode located on the outside of the Blood Collection Kit. Scanning it automatically captured a unique, eight-digit code, thereby linking the respondent to the transport tubes / labels within it, the corresponding ODK questionnaire data, and ultimately to LCBR results.

Following standard phlebotomy protocols, FEs asked respondents to identify an arm for collecting blood, applied the tourniquet to that arm, and identified a vein in the antecubital fossa for venipuncture. If no vein appeared suitable, FEs asked to try the opposite arm. Unless respondents had objections, venipuncture was performed on the best potential vein and whole blood was collected, as summarized below:

- Put on nitrile gloves.
- Have the respondent extend his/her arm on the protective pad, palm up and straight at the elbow.
- Inspect the arm. Do not draw blood from an arm that has a rash, open sore, is swollen or shows signs of a recent venipuncture or hematoma. Do not draw blood from an arm that contains an arterial access such as a fistula or shunt.
- Apply the tourniquet several inches above the elbow and palpate for a suitable vein.
- Select a vein that is palpable and well-fixed to surrounding tissue.
- Open the needle assembly unit and attach it to the vacutainer holder.
- Ask the respondent to make a tight fist. Cleanse the area with an alcohol wipe using a circular motion and allow the area to air dry.
- Remove the cover from the needle.
- The vein should be fixed or held taut during the puncture. Push the needle firmly and deliberately into the vein. When firmly in the vein, blood appears in the tubing of the needle assembly past the end of the needle.
- Attach the needle holder and quickly push the first vacutainer tube (ordered in Exhibit 2, below) onto the needle in the holder, puncturing the center of the stopper.
- Release the tourniquet after the flow is established or if the respondent becomes uncomfortable. The respondent may open his/her fist once blood flow is established.
- When the first vacutainer tube is filled to capacity, remove it from the holder and place the next vacutainer tube in the holder.
- Gently invert each vacutainer tube 8-10 times immediately upon removing each one and while filling the next one. Repeat until all the desired vacutainer tubes are filled.
- Place all filled vacutainer tubes directly into a cooler with ice or ice packs.
- When the last vacutainer tube is filled, remove the tourniquet, carefully withdraw the needle, and cover the venipuncture site with a sterile gauze pad.
- Never apply pressure to the gauze until the needle is clear of the puncture site and away from the arm.
- Have the respondent hold the gauze pad with mild pressure and sit quietly for a few minutes.

- Slide the needle safety guard forward to prevent an accidental needle stick. Discard the entire used needle assembly in a sharps container.
- Check the venipuncture site. If it is adequately clotted, remove the gauze and apply a bandage. If after a few minutes, bleeding continues keep direct pressure on the site for 5 minutes.
- Encourage the respondent to sit quietly for a few minutes. Due to a fasting blood draw encourage the respondent to eat a snack if needed.

When the first attempt at blood collection was unsuccessful, FEs were allowed to ask to draw blood from the opposite arm. However, no more than two blood collection attempts were permitted. Moreover, only the antecubital fossa was acceptable for blood draw. FEs were not allowed to collect blood from any other sites, such as the back of the hand.

Either 5 or 6 tubes of blood were collected per respondent, depending on eligibility for a separate glucose sub-study (see Measures of Glucose Homeostasis User Guide, Section 4.1.2.1). Collection order, tube type, and processing information are listed below (Exhibit 2).

Order	Tube Type	Centrifuged	Resultant supernatant	Resultant precipitate	Use
1	8.5 ml SST	Yes	Serum	Discarded	Assays: glucose, total cholesterol, high- & low-density lipoprotein-cholesterol, triglycerides, high sensitivity C reactive protein, creatinine & cystatin C
2	10 ml EDTA	Yes	Plasma	RBC/buffy coat	Archival: for future use
3	3 or 4 ml EDTA	No	N/A	N/A	Assay: hemoglobin A1c Archival: for future use
4	8.5 ml SST	Yes	Serum	Discarded	Archival: for future use
5	6 ml NaFl/KOx	Yes	Plasma	Discarded	Assay: glucose sub-study
6	10 ml PAXgene	No	N/A	N/A	Archival: for future use

Exhibit 2. Tubes of Blood Collected

3.3.2 Blood Processing

The venous blood draw concluded the home exam. After cleaning up all supplies and equipment, FEs left the exam sites and were allowed a maximum of two hours before processing the blood which was chilled at 4° C (on ice or frozen cold packs) in the interim.

All FEs centrifuged specific blood collection tubes, including the 8.5 ml SST, 10 ml EDTA, and when collected, the 6 ml NaFl/KOx vacutainer tubes. The 3-4 ml EDTA vacutainer tube used for the HbA1c assay was *not* centrifuged. FEs centrifuged tubes for ≥ 10 min at ≥ 1300 g, depending on the capabilities of their centrifuge. After centrifugation, FEs used the graduated transfer pipettes included in the Blood Collection Kit to aliquot serum from the SST and (separately, when collected) plasma from the NaFl/KOx vacutainer tubes into 10 ml, round bottom, skirted transport tubes (BD Biosciences, NJ). FEs aliquoted as much supernatant as possible into the transport tubes but avoided disturbing the precipitate layer. A red cap identified transport tubes containing serum from the SST vacutainer tubes, a blue cap identified transport tubes containing plasma from the 10 ml EDTA vacutainer tube, and a white cap identified the transport tube containing plasma from the Na/Fl/KOx tube. Transport tubes were chilled at 4° C (on ice or frozen cold packs) until packaged for shipment to LCBR. Exhibit 3 demonstrates the complete blood processing protocol.

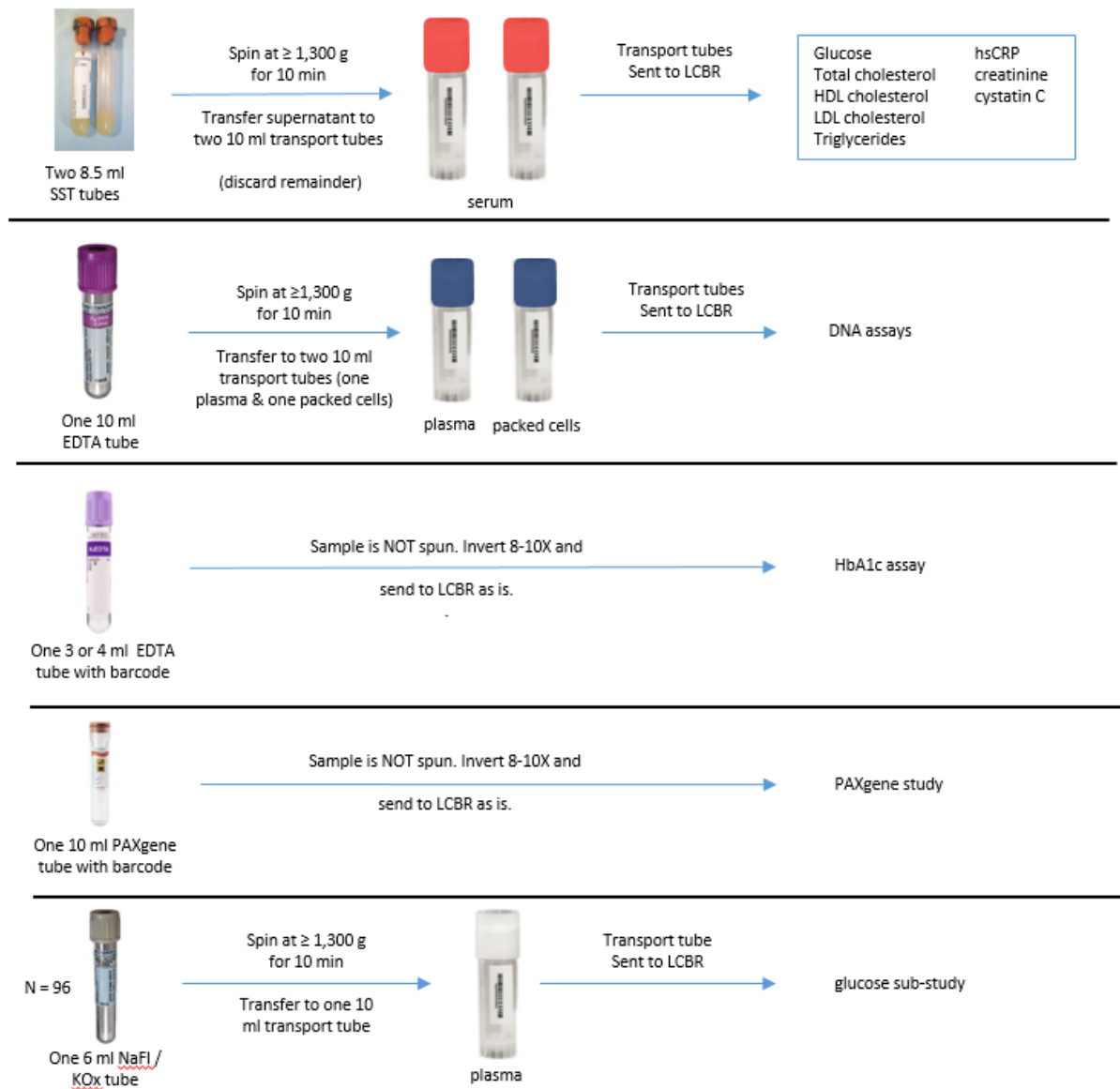


Exhibit 3. Blood Processing Protocol

After processing the blood, FEs took a loose barcode label provided in the Blood Collection Kit and affixed it to a paper manifest designed to accompany the transport tubes to LCBR. The loose barcode label matched the barcode labels on the transport tubes and the Shipping Box. FEs recorded all vacutainer tubes that were collected and identified all difficulties during blood draw or processing on the manifest as well as in the tablet. The barcode-labelled manifest was designed to be scanned on arrival at LCBR to associate it with an individual respondent’s transport tubes.

3.3.3 Shipment of Samples

Immediately before shipment, FEs removed two cold packs from the freezer, sandwiched the transport tubes between them, enclosed the sandwich within the Styrofoam Box, placed the manifest on top of

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the Styrofoam Box, sealed the cardboard Shipping Box around it, put the cardboard Shipping Box inside the Tyvek envelope, applied a pre-printed FedEx shipping label to the envelope, carried it to a FedEx office, and handed it to a FedEx representative (*in person*) for Priority Overnight shipment to LCBR with arrival the following morning. FEs were not permitted to leave shipments at unattended FedEx drop boxes.

When overnight shipment was impossible, FEs noted this on the manifest and held unboxed transport tubes in a refrigerator approved for biological specimens or cooler with enough cold packs to keep them chilled at 4° C overnight without risk of freezing (or thawing), as is possible on wet or dry ice. The transport tubes were packaged and shipped the next day using freshly frozen cold packs.

3.3.4 Receipt of Samples at LCBR

LCBR technicians specifically trained for Add Health Wave V received and immediately processed samples each morning. They unpacked the shipping boxes one at a time, evaluated the volume and quality of each transport tube, and entered them into a custom-made laboratory information management system (LIMS) program.

After re-centrifuging the serum samples for renal biomarker assays at 4° C for 10 min at 30,000 g, the technicians aspirated the supernatant, discarded all remaining precipitate, transferred the aspirate to pre-labelled tubes, and placed them in a biospecimen refrigerator for archival (in 1 ml aliquots at -80° C) or assay (500 ul aliquot). The LCBR technicians entered all aliquot information into the LIMS system.

Samples for the creatinine assay were transferred to a pre-labelled tube for a 5-10-minute cold transport via a daily 3:00 pm courier to Pathology and Laboratory Medicine, in the University of Vermont Medical Center (UVMCC) for analysis. A separate 200 µl aliquot of serum was made for the cystatin C assay, which was run in-house at LCBR. This sample was kept chilled until the time of the assay. All assays were performed on the same day that the samples arrived at LCBR.

4. Assay and Internal Quality Control

4.1 Creatinine [H5CREAT]

4.1.1 Rationale

Creatinine is a waste product derived from the normal breakdown of muscle in the body. As it is produced, it is filtered through the glomeruli of the kidneys. Serum creatinine concentrations are therefore instrumental in determining normal kidney function and can be increased in acute or chronic renal failure, urinary tract obstruction, reduced renal blood flow, shock, dehydration, and rhabdomyolysis.

4.1.2 Colorimetric Assay Protocol

All creatinine assays were run on the same day of sample arrival at LCBR using an Ortho VITROS 5600 Integrated System (Ortho Clinical Diagnostics, Raritan, NJ) and VITROS Chemistry Products CREA slides,

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i.e. multilayered, analytical elements coated on a polyester support (see Exhibit 4). Serum from venous blood collected using the SST vacutainer tubes was introduced into the VITROS system by placing sample vials holding 500 µl of serum into an automatic sampling tray, after which all processes were automatically performed and results output by the VITROS system.

The VITROS system read barcodes on the vials to automatically determine which assays to run. In addition to creatinine, other assays were run from the same serum sample, including total cholesterol, high-density lipoprotein cholesterol, triglycerides, and glucose. Only the creatinine assay is described below. Assay protocols for other analytes can be found in other Add Health User Guides.

<p>Slide Ingredients</p>	<p style="text-align: center;">Slide Diagram</p>	<ol style="list-style-type: none"> 1. Upper slide mount 2. Spreading layer (TiO₂) 3. Reagent layer <ul style="list-style-type: none"> • creatinine amidohydrolase • creatine amidohydrolase • sarcosine oxidase • peroxidase • leuco dye • buffer, pH 7.0 4. Support layer 5. Lower slide mount
<p>Reactive Ingredients per cm²</p> <p>Creatinine amidohydrolase (<i>Flavobacterium sp.</i>, E.C.3.5.2.10) 0.20 U; creatine amidohydrolase (<i>Flavobacterium sp.</i>, E.C.3.5.3.3) 4.7 U; sarcosine oxidase (<i>Bacillus sp.</i>, E.C.1.5.3.1) 0.55 U; peroxidase (horseradish root, E.C.1.11.1.7) 1.6 U and 2-(3,5-dimethoxy-4-hydroxyphenyl)-4,5-bis(4-dimethylaminophenyl)imidazole (leuco dye) 32 µg.</p> <p>Other Ingredients</p> <p>Pigment, binders, surfactants, stabilizer, scavenger, chelator, buffer, dye solubilizer and cross-linking agent.</p>		

Exhibit 4. Ortho-Vitros CREA slide

Upon introduction of each vial into the analyzer, 40 µl of serum was aspirated, deposited onto a CREA slide for analysis, and evenly distributed by the spreading layer to the underlying layers. Creatinine diffused to the reagent layer, where it was hydrolyzed to creatine in the rate-determining step. The creatine was converted to sarcosine and urea by creatine amidohydrolase. The sarcosine, in the presence of sarcosine oxidase, was oxidized to glycine, formaldehyde, and hydrogen peroxide. The final reaction involved the peroxidase-catalyzed oxidation of a leuco dye to produce a colored product³.

Following the addition of the sample, the slide was incubated. During the initial reaction phase, endogenous creatine in the sample was oxidized. The resulting change in reflection density at a wavelength of 670 nm was measured at 2 time points, 5 minutes apart. The difference in reflection density was determined using the software-resident two-point rate math model and was proportional to the concentration of creatinine present in the sample. The specific reaction scheme is listed in Exhibit 5.

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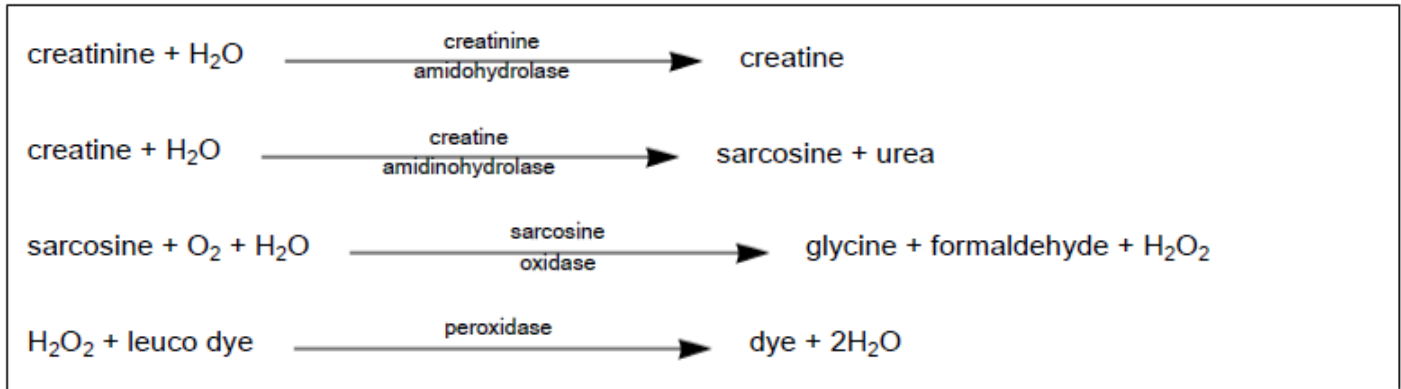


Exhibit 5. Creatinine Assay Reaction Scheme

Once the assay was carried out, the reflected light output was compared to a standard curve generated by the use of a VITROS Chemistry Products Calibrator Kit 1 (Ortho Clinical Diagnostics, Raritan, NJ). The concentrations were output to a Sunquest computer system (Sunquest Information Systems, Tucson AZ) that linked the UVMCM data with LCBR's LIMS system.

The VITROS 5600 system's dynamic reporting range of the creatinine assay was from 0.05 – 14.0 mg/dL. When concentrations exceeded the upper limit, the VITROS system automatically diluted the samples 1:2 with a VITROS Chemistry Products FS Diluent Pack 2 (Ortho Clinical Diagnostics, Raritan, NJ) until the concentrations were within range. Any dilutions were reported along with the creatinine concentrations in the assay results. Dilutions and creatinine concentrations that accounted for the reflexive dilutions via multiplication by the dilution factor were reported simultaneously. The final creatinine concentrations (**H5CREAT**) ranged from 0.36 to 14.57 mg/dL.

4.1.3 Internal Quality Control

The Ortho-VITROS system was maintained daily by cleaning machine components, replacing all reagents, and running known quality control samples (Ortho Clinical Diagnostics, Raritan, NJ). Internal quality controls consisted of VITROS Performance Verifier I, which was typically 0.70-1.0 mg/dL creatinine, and VITROS Performance Verifier II, which was typically 5.0-6.0 mg/dL creatine, depending on the specific lot used.

The values assigned to the VITROS Chemistry Products Calibrator Kit 1 for creatinine are traceable to a Gas Chromatography Isotope Dilution Mass Spectrometry (GC/IDMS) method ⁴ and National Institute of Standards and Technology (NIST) SRM[®] 914 creatinine standard reference material.

In addition to the daily quality control, LCBR used two pools of samples from twenty normal donors (US Biologicals, Salem, MA) in longitudinal quality control analyses. One pool was an EDTA plasma normal donor pool (Lot #E050115). The other pool was a serum normal donor pool (Lot #S042715). LCBR periodically assayed both pools over the course of Wave V. The plasma and serum creatinine concentration means and coefficients of variation based on those assays were 0.92 mg/dL (2.78%) and

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0.95 mg/dL (2.17%), respectively. When creatinine concentrations exceeded acceptable parameters, the Ortho-VITROS system was investigated and repaired.

4.2 Cystatin C [H5CYSC]

4.2.1 Rationale

Cystatin C is a non-glycosylated 13-kDa protein and is a member of the cystatin superfamily of cysteine protease inhibitors. Compared to serum creatinine, the level of serum cystatin C is not significantly affected by age, sex, race, or muscle mass.⁵ In addition, cystatin C is almost freely filtered through the glomerular membrane and then nearly completely reabsorbed and degraded by the proximal tubular cells. Therefore, serum levels of cystatin C are almost exclusively determined by the glomerular filtration rate (GFR), making cystatin C an excellent indicator for GFR.⁶

4.2.2 Colorimetric Assay Protocol

All cystatin C assays were run on the same day as sample arrival using a Siemens BNII / BN Prospec System (Siemens Healthcare Diagnostic Products GmbH, Marburg, Germany) and a cystatin C specific particle enhanced immunonephelometric assay. In this assay, polystyrene particles coated with specific antibodies to cystatin C were aggregated when mixed with Add Health serum samples containing cystatin C. These aggregates scattered a beam of light that was passed through the sample. The intensity of the scattered light was proportional to the concentration of the respective protein in the sample. The result was then evaluated by comparison with a standard of known concentration.⁷ Serum from venous blood collected using the SST vacutainer tubes was introduced into the Siemens system by placing sample vials holding 200 µl of serum into an automatic sampling tray, after which all processes were automatically performed and results output by the Siemens system.

The Siemens system read barcodes on the vials to automatically determine which assay to run. Upon introduction of each vial into the analyzer, 40 µl of serum was aspirated and used for the assay. All samples were automatically diluted 1:100 with N diluent OUMT65 (Siemens Healthcare, Newark, DE).

The Siemens system's dynamic reporting range of the cystatin C assay was determined by a generated reference curve and multi-point calibration using two control level reagents and a Protein Standard. N Cystatin C Control Level 1 and 2 were calibrated to protein standard preparations of Siemens Healthcare Diagnostics Products GmbH and were lot dependent. Typical values (and coefficient of variation) were 0.9 mg/L (2.5%) for Control 1 and 1.8 mg/L (2.3%) for Control 2. Serial dilutions of the N Protein Standard UY were automatically prepared by the Siemens system and compared to the reference curve. The typical reporting range was between 0.25-8.1 mg/L using a sample dilution of 1:100. When sample concentrations exceeded the upper limit, the Siemens system automatically diluted the samples with 20 µl of N diluent solution until the concentrations were within range. Dilutions and cystatin C concentrations that accounted for the reflexive dilutions via multiplication by the dilution factor were reported simultaneously.

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4.2.3 Internal Quality Control

The Siemens system was maintained daily by inspecting all tubing, syringes, and connections for leaks, cracks, or contamination. N diluent solution, reaction buffers, and wash solutions were also changed daily.

N Cystatin C Control Levels 1 and 2 were assayed after each establishment of a reference curve, the first use of a reagent vial as well as with each run of samples. LCBR also ran EDTA and serum controls as specimen samples each time a new standard curve was established. In addition to the daily quality control, LCBR used two pools of samples from twenty normal donors (US Biologicals, Salem, MA) in longitudinal quality control analyses. One pool was an EDTA plasma normal donor pool (Lot #E050115). The other pool was a serum normal donor pool (Lot #S042715). LCBR periodically assayed both pools over the course of Wave V. The plasma and serum cystatin C concentration mean and coefficient of variation based on those assays was 0.713 mg/L (7.41%) and 0.654 mg/L (8.10%) before International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) standardization and 0.840 mg/L (3.68%) and 0.79 mg/L (3.85%) after IFCC standardization (see sections 4.2.4-4.2.5). When cystatin C concentrations exceeded acceptable parameters, the Siemens system was investigated and repaired.

4.2.4 Standardization

In 2010, The IFCC Working Group for the Standardization of Cystatin C (WG-SCC), in collaboration with the Institute for Reference Materials and Measurements (IRMM), announced the availability of the new certified cystatin C reference material, ERM-DA471/IFCC.⁸ Although use of this reference standard had progressed in Europe since the announcement, only recently had Food and Drug Administration approved it for use in the United States.⁹ Following approval, Siemens notified LCBR in March 2018 that it was discontinuing the pre-IFCC standardization N Latex Cystatin C Reagent Assay Kit because the FDA had approved the ERM-DA471/IFCC reference material. Thereafter, assay kits using the post-IFCC standardization N Latex Cystatin C became available to U.S. users for measuring IFCC-calibrated cystatin C concentrations. Between April 2016 and November 2017, LCBR had already run 1940 Add Health respondent samples, but did not have enough pre-IFCC assay kits to process the remainder. Moreover, Siemens did not have any more pre-IFCC assay kits.

4.2.5 Inter-conversion of Pre- & Post-IFCC Standardization of Cystatin C Concentrations

Four samples were therefore selected at random from those 1940 respondents with pre-IFCC standardization cystatin C concentrations within each of 24 strata defined by race/ethnicity (non-Hispanic white; non-Hispanic black; Hispanic; other), sex (male; female), and cystatin C concentration ventile (1st; 2nd-19th; 20th), i.e. < 0.5, 0.5-0.8, and > 0.8 mg/L. Collectively, the stratified, random sample included $4 \times 24 = 96$ respondents among whom archived 1 ml frozen serum vials were thawed, pre-IFCC standardization cystatin C concentrations were re-assayed in 40 μ l, all pre-IFCC reagents and standards were replaced with the post-IFCC set, the Siemens BNII system was recalibrated, post-IFCC standardization cystatin C concentrations were assayed in another 40 μ l maintained at 4°C in the interim, and the remaining 920 μ l of serum were re-archived. The association between the post- and pre-IFCC standardization cystatin C concentrations (Exhibit 6) was modeled using Deming regression,^{10,11} i.e. post-IFCC cystatin C = $\alpha + \beta \times$ pre-IFCC cystatin C (Pearson $r = 0.98$). Estimates of α , β and their 95% CIs were 0.003 (-0.057, 0.063) and 1.164 (1.077, 1.251). Because α was small and no different from zero,

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β was used to convert all pre- to post-IFCC cystatin C concentrations via multiplication and converted concentrations were flagged accordingly (**H5CYSCAJ=1**). The final post-IFCC cystatin C concentrations (**H5CYSC**) ranged from 0.34 to 8.08 mg/L.

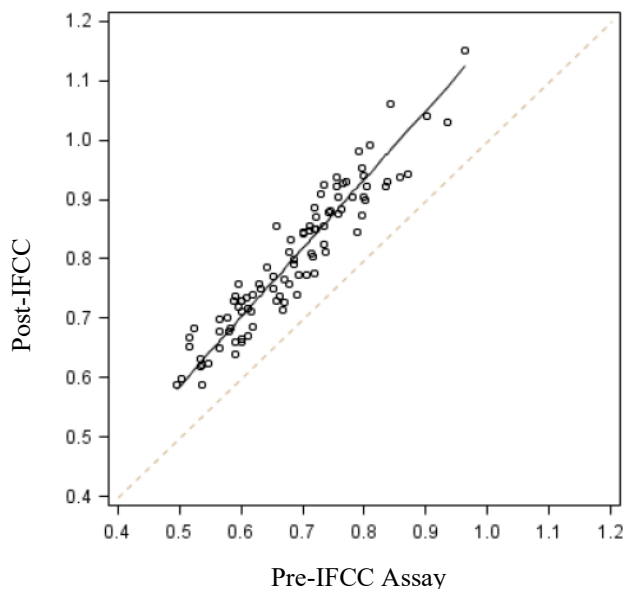


Exhibit 6. Plot of Post- Versus Pre-IFCC Standardized Cystatin C Concentrations (mg/L)

5. External Quality Control

5.1. Reliability

Within a race/ethnicity- and sex-stratified random sample of 103 Add Health respondents among whom venous blood was collected twice, on average 14.3 (95% confidence interval: 13.0-15.5) days apart, typically by the same FE and at approximately the same time of day, the reliability of creatinine (mg/dL) and cystatin C (mg/dL) was estimated as an intra-class correlation coefficient (ICC, 95% confidence interval). The resulting estimates (Exhibit 7) suggest that the home exam venous blood yielded highly reliable creatinine and cystatin C concentrations.

Measure	n	ICC	95% CI
Creatinine (mg/dL)	103	0.93	(0.91, 0.96)
Cystatin C (mg/L)	103	0.86	(0.81, 0.91)

Exhibit 7. Reliability of Creatinine and Cystatin C

6. Constructed Measures

6.1. Estimated Glomerular Filtration Rate (eGFR) According to NIDDK CKD-EPI Guidelines [H5GFRCRE; H5GFRCYC; H5GFRCC]

The glomerular filtration rate (GFR, ml/min/1.73 m²) was estimated in three different ways using either the creatinine concentration, the cystatin C concentration or both concentrations. Each was estimated using the following National Institute of Diabetes and Digestive and Kidney Diseases Chronic Kidney Disease Epidemiology Collaboration (NIDDK CKD-EPI) equations:¹

- Based on creatinine (**H5GFRCRE**)

$$\text{eGFR}_{\text{cr}} = 141 \times \min(\text{Scr}/\kappa, 1)^{\alpha} \times \max(\text{Scr}/\kappa, 1)^{-1.209} \times 0.993^{\text{Age}} \\ [\times 1.018 \text{ if female}] [\times 1.159 \text{ if black}]$$

where:

- Scr = creatinine concentration (mg/dL)
- $\kappa = 0.7$ (female) or 0.9 (male)
- $\alpha = -0.329$ (female) or -0.411 (male)

- Based on cystatin C (**H5GFRCYC**)

$$\text{eGFR}_{\text{cys}} = 133 \times \min(\text{Scys}/0.8, 1)^{-0.499} \times \max(\text{Scys}/0.8, 1)^{-1.328} \times 0.996^{\text{Age}} \\ [\times 0.923 \text{ if female}]$$

where:

- Scys = cystatin C concentration (mg/L)

- Based on creatinine and cystatin C (**H5GFRCC**)

$$\text{eGFR}_{\text{cr_cys}} = 135 \times \min(\text{Scr}/\kappa, 1)^{\alpha} \times \max(\text{Scr}/\kappa, 1)^{-0.601} \times \\ \min(\text{Scys}/0.8, 1)^{-0.375} \times \max(\text{Scys}/0.8, 1)^{-0.711} \times 0.995^{\text{Age}} \\ [\times 0.969 \text{ if female}] [\times 1.080 \text{ if black}]$$

where:

- Scr = creatinine concentration (mg/dL)
- Scys = cystatin C concentration (mg/L)
- $\kappa = 0.7$ (female) or 0.9 (male)
- $\alpha = -0.248$ (female) or -0.207 (male)

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6.2. Classifications of eGFR According to KDIGO Guidelines [H5CGFRKR; H5CGFRKY; H5CGFRKC]

The following classification variables were constructed according to the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines:²

- Based on creatinine [H5CGFRKR]
- Based on cystatin C [H5CGFRKY]
- Based on both creatinine and cystatin C [H5CGFRKC]

Each variable includes the six classifications shown in Exhibit 8.

eGFR		
Classification	(ml/min/1.73 m ²)	KDIGO Class
1	≥ 90	Normal/High (G1)
2	60-89	Mildly decreased (G2)
3	45-59	Mildly to moderately decreased (G3a)
4	30-44	Moderately to severely decreased (G3b)
5	15-29	Severely decreased (G4)
6	< 15	Kidney failure (G5)

Exhibit 8. KDIGO Classification of eGFR based on Creatinine, Cystatin C, or Both

6.3. Clinical Classifications of eGFR 6.3 [H5CGFRCR; H5CGFRCY; H5CGFRCC]

The following classification variables were constructed:

- Based on creatinine [H5CGFRCR]
- Based on cystatin C [H5CGFRCY]
- Based on both creatinine and cystatin C [H5CGFRCC]

Each variable includes the three classifications shown in Exhibit 9.

GFR		
Classification	(ml/min/1.73 m ²)	Clinical Class
1	≥ 60	Neither
2	15-59	Chronic kidney disease (CKD)
3	< 15	End-stage kidney disease (ESKD)

Exhibit 9. Clinical Classification of eGFR based on Creatinine, Cystatin C, or Both

7. The Renal Function Data File (brenal5.xpt)

7.1. Structure

The structure of the disseminated renal function data file is flat. This means that it is a respondent-level data file, where each respondent has one and only one record. The respondent's identifying number (the AID variable) will appear in the data file only once.

7.2. Contents

The renal function data file includes the variables below, which are described in the corresponding codebook documentation that also contains frequencies.

<u>Variable Name</u>	<u>Variable Description</u>
AID	Respondent Identifier
H5CREAT	Creatinine (mg/dL)
H5CYSC	Cystatin C (mg/L)
H5CYSCAJ	Flag: Cystatin C converted from pre- to post-IFCC standardization
H5GFRCRE	eGFR Creatinine (ml/min/1.73 m ²)
H5GFRCYC	eGFR Cystatin C (ml/min/1.73 m ²)
H5GFRCC	eGFR Creatinine & cystatin C (ml/min/1.73 m ²)
H5CGFRKR	eGFR Creatinine classification according to KDIGO
H5CGFRKY	eGFR Cystatin C classification according to KDIGO
H5CGFRKC	eGFR Creatinine & cystatin C classification according to KDIGO
H5CGFRCR	eGFR Creatinine clinical classification
H5CGFRCY	eGFR Cystatin C clinical classification
H5CGFRCC	eGFR Creatinine & cystatin C clinical classification
H5Q045D	Q045d Ever diagnosed with kidney disease or failure

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