2025

Add Health Wave VI Documentation

Add Health

The National Longitudinal Study of Adolescent to Adult Health

Measures of Glucose Homeostasis User Guide

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

- Anthropometrics
- Baroreflex Sensitivity & Hemodynamic Recovery
- Biomarker Weights
- Cardiovascular Measures
- Hepatic Injury
- Home Exam – Medication Use
- Home Exam Questionnaire and QC Metrics
- Infection
- Inflammation and Immune Function
- Lipids
- Neurodegeneration
- Renal Function

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1. Introduction

Wave IV measures of glucose homeostasis were based on dried blood spots collected using capillary finger prick.[1] In contrast, Wave V-VI measures of glucose homeostasis were based on venous blood collected via phlebotomy. Assay methods at Wave V[2] also differed from those at Wave VI, as highlighted here and below. The 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 Glucose Concentrations

- Glucose (mg/dl)
- Hemoglobin A1c (HbA1c, %)

Moreover, the restricted use Add Health Wave VI data include six constructed measures:

- Fasting status (h)
- Classification of fasting glucose[3]
- Classification of non-fasting glucose[3]
- Classification of HbA1c [3]
- Anti-diabetic medication use
- Joint classification of glucose, HbA1c, self-reported history of diabetes, and anti-diabetic medication use

2. General Overview of Data Collection

All Wave VI venous blood samples were collected during home exams performed by ExamOne, a subsidiary of Quest Diagnostics®. All FEs were trained and certified using a custom program specific to the Add Health protocol. FEs used a 7" Samsung Galaxy Tab A7 Lite 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.

FEs conducted home exams among previously consented participants. 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 participant. All participants had the option to decline part or all the blood draw, although declining did not affect their ability to participate in the rest of the home exam. Overall, 90.8% of the participants agreed to and completed the blood draw. Of the remainder, 6.4% refused, 2.1% agreed but the blood draw was unsuccessful, and < 1% had exams terminated before the blood draw (see the blood draw status variable H6BLOOD in the *bdemo6* 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^{o} 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 participant. 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 a.m. 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 pre-specified biomarkers of metabolic, hepatic, renal, amyloid-tau-neurodegenerative (ATN), inflammatory, immune, and infectious conditions, including the measures of glucose homeostasis 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 (**Figure 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) 3 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 vacutainer tubes, and transport tubes were supplied by Simport Scientific (Quebec, Canada).

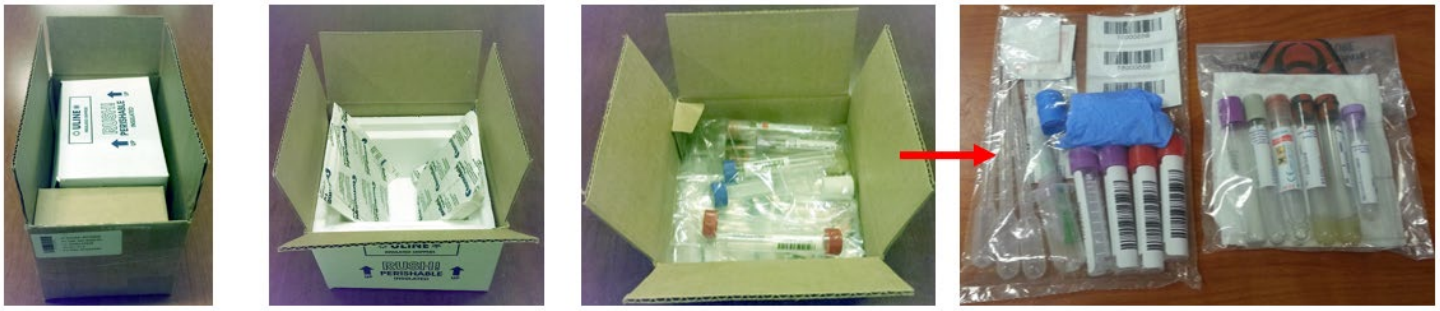

Figure 1. Visit Supply and Blood Collection Kits

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 participants were comfortable giving blood, participants were asked to either sit or recline at their discretion. They also 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 and on all vacutainer tubes. Scanning it automatically captured a unique, eight-digit code, thereby linking the participant to the transport tubes / labels within the kit, the corresponding ODK questionnaire data, and ultimately to LCBR results.

Following standard phlebotomy protocols, FEs asked participants 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 participants had objections, venipuncture was performed on the best potential vein and whole blood was collected, as summarized below:

- Put on nitrile gloves.
- Have the participant 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 participant 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 Figure 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 participant becomes uncomfortable. The participant 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 participant 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 participant to sit quietly for a few minutes. Due to a fasting blood draw encourage the participant 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.

5 tubes of blood were collected per participant. Collection order, tube type, and processing information are listed below (**Figure 2**).

Order	Tube Type	Centrifuged	Resultant supernatant	Resultant precipitate	Use
1	8.5 ml SST	Yes	Serum	Discarded	Assay: glucose, total cholesterol, high- & low-density lipoprotein-cholesterol, triglycerides, AST, ALT, creatinine, hsCRP, IL-1β, IL-6, IL-8, IL-10, TNFα, CMV, HSV, SARS CoV-2 (RBD; spike; nucleocapsid) IgG
2	10 ml EDTA	Yes	Plasma	Packed cells	Assay: Neurofilament light, Tau, GFAP. Archival: packed cells for future use
3	3 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	10 ml PAXgene	No	N/A	N/A	Archival: for future use

Figure 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^{o} C (on ice or frozen cold packs) in the interim.

All FEs centrifuged the 8.5 ml SST and 10 ml EDTA vacutainer tubes. The 3 ml EDTA vacutainer tube used for the HbA1c assay and the PAXgene tube were *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 tubes and plasma/packed cells from the 10 ml EDTA 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 and a blue cap identified transport tubes containing plasma from the 10 ml EDTA vacutainer tube. Transport tubes were chilled at 4^{o} C (on ice or frozen cold packs) until packaged for shipment to LCBR. **Figure 3** demonstrates the complete blood processing protocol.

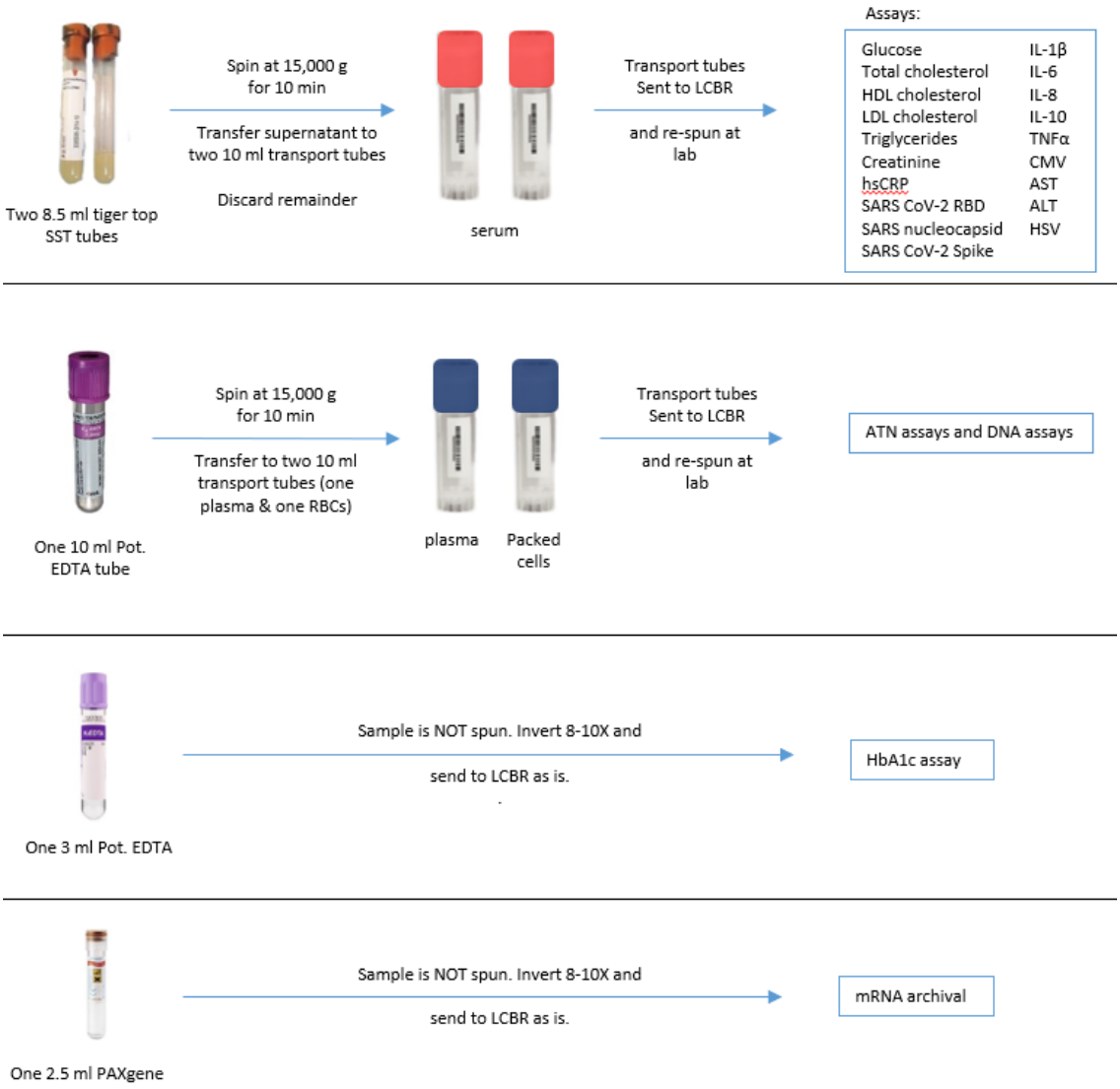

Figure 3. Blood Processing Protocol

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 participant's transport tubes.

3.3.3 Shipment of Samples

Immediately before shipment, FEs removed two cold packs from the freezer, placed the transport tubes in a sleeve, sandwiched the transport tubes between the ice packs, enclosed the sandwich within the Styrofoam box, placed the manifest on top of 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^{o} 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 VI 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 glucose 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.

The LCBR technicians also remixed the vacutainer tube used to collect and transport the venous whole blood sample for HbA1c assay, then aspirated and archived a 1 ml aliquot for future use. 350 µl of the remaining sample was then 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 (UVMMC) for analysis.

4. Assay and Internal Quality Control

4.1 Glucose [H6GLUCOS]

4.1.1 Rationale

Serum glucose was assayed because of its central role in the identification of impaired fasting glucose / pre-diabetes, and diabetes mellitus, two well-known risk factors for cardiovascular disease morbidity and mortality.

4.1.2 Colorimetric Assay Protocol

All glucose 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 GLU slides, i.e. multilayered, analytical elements coated on polyester supports (**Figure 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 glucose, other assays were run from the same serum sample, including total cholesterol, high-density lipoprotein cholesterol, triglycerides, AST, ALT, and creatinine. Only the glucose assay is described below. Assay protocols for other analytes can be found in other Add Health User Guides.

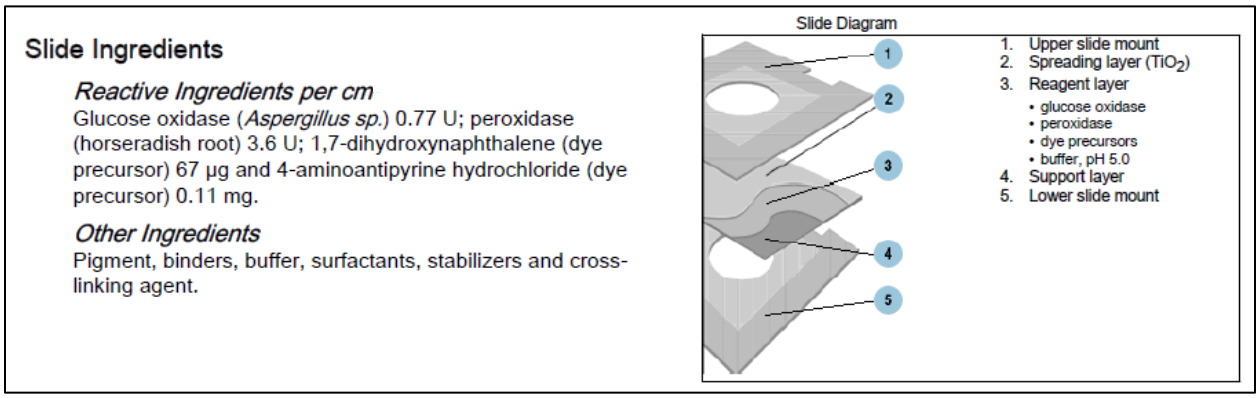

Figure 4. Ortho-Vitros GLU Slide

Upon introduction of each vial into the analyzer, 10 µl of serum was aspirated, deposited onto a GLU slide for analysis, and evenly distributed by the slide's spreading layer to the underlying layers. The oxidation of serum glucose was catalyzed by glucose oxidase to form hydrogen peroxide and gluconate. This reaction was followed by an oxidative coupling catalyzed by peroxidase in the presence of dye precursors to produce a dye[4]. The intensity of the dye was then measured by reflected light. The specific reaction scheme is displayed in **Figure 5**.[5]

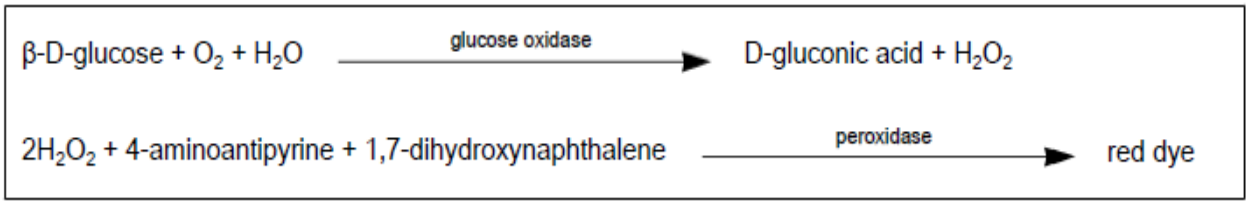

Figure 5. Glucose Assay Reaction Scheme

The intensity of the dye was measured and corresponding glucose concentration inferred at room temperature by comparing reflected light output at 540 nm to a standard curve generated using a VITROS Chemistry Products Calibrator Kit (Ortho Clinical Diagnostics, Raritan, NJ).[6] The concentrations were output to a Sunquest computer system (Sunquest Information Systems, Tucson AZ) that linked the UVMMC data with LCBR's LIMS system.

The VITROS 5600 system's dynamic reporting range of the glucose assay was 20-625 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 (Ortho Clinical Diagnostics, Raritan, NJ) until the concentrations were within range. Dilutions and glucose concentrations that accounted for the reflexive dilutions via multiplication by the dilution factor were reported simultaneously. The final glucose concentrations (H6GLUCOS) ranged from 30 to 533 mg/dl. Glucose values less than 40 mg/dl were considered implausible and assigned a code of -9999 in the dataset.

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 (Thermo Fisher Scientific, Waltham, MA). Internal quality controls consisted of MAS OmniCORE™ quality controls. Several lots (OCR2406, OCR2511, OCR2608) were used throughout Wave VI, but all lots had similar known control values. For GLU, the low, middle, and high control values typically ranged from 50-60, 180-220, and 315-380 mg/dl respectively.

Values assigned to the VITROS Chemistry Products Calibrator Kit for glucose are traceable to the Certified NIST (National Institute of Standards and Technology) Reference Material, SRM® (Standard Reference Material) 917. The Ortho-Clinical Diagnostics calibration laboratory uses SRM® 917 to

calibrate the CDC Hexokinase method to support glucose concentration assignment for VITROS Calibrator Kit 1.

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 #E011221). The other pool was a serum normal donor pool (Lot #S120419). LCBR periodically assayed both pools over the course of Wave VI. The plasma and serum glucose concentration mean (coefficient of variation) based on those assays was 106 mg/dl (1.8%) and 82 mg/dl (2.0%), respectively. When glucose concentrations exceeded acceptable parameters, the Ortho-VITROS system was investigated and repaired.

4.2 Hemoglobin A1c (HbA1c) [H6HBA1C]

4.2.1 Rationale

HbA1c was assayed in venous whole blood because it is an integrated measure of glucose homeostasis, reflecting average blood glucose over the preceding two to three months. The measure plays a critical role in the management of diabetes since it is correlated with micro- and macrovascular complications and is widely used as the standard biomarker for the adequacy of glycemic management.[3]

4.2.2 Capillary Electrophoresis Protocol

HbA1c assays were run daily by LCBR. Samples received at LCBR were stored at 4°C in sample refrigerators until they were delivered to UVMMC with all other samples for that day. Assays were performed on a Capillarys 3 Tera instrument using a Sebia CAPI 3 HbA1c Assay kit (Norfolk, GA) including buffer solution, hemolyzing solution, calibrators, and control samples. The CAPILLARYS 3 instrument uses the principle of capillary electrophoresis in free solution. With this technique, charged molecules were separated by their electrophoretic mobility in an alkaline buffer with a specific pH. Separation also occurred according to the electrolyte pH and electroosmotic flow. The CAPILLARYS 3 instrument has silica capillaries functioning in parallel allowing 12 simultaneous analyses for HbA1c quantification in a whole blood sample. A sample dilution with hemolyzing solution was prepared and injected by aspiration at the anodic end of the capillary. A high voltage protein separation was then performed and direct detection of the hemoglobins was made at the cathodic end of the capillary at 415 nm, which is the absorbance wavelength specific to hemoglobins. Before each run, the capillaries were washed with a wash solution and prepared for the next analysis with buffer. Direct detection provided accurate relative quantification of the hemoglobin A1c fraction.[7]

1000 µl of whole blood was allowed to warm to room temperature, vortexed, and placed into sample tube racks which were then inserted into the Capillarys 3 instrument. Each rack was identified by barcode scanning, and then all sample tubes were mixed, diluted with hemolyzing solution, and the

diluted samples were injected into the capillaries. Voltage was then applied to the capillaries for 9 minutes and samples were maintained at a constant temperature. Hemoglobins were then detected by scanning at 415 nm and the results were output to the data processing software. Relative quantification of individual HbA1c fractions was performed automatically, standardized, and reported in percentages and in mmol/mol according to IFCC recommendations.[8] The Capillarys 3 system's dynamic reporting range of the HbA1c assay was 4.0% - 16.5% (20 - 157 mmol/mol). Note that HbA1c (mmol/mol) can be converted to HbA1c (%), as follows:

$$HbA1c\ (\%) = [HbA1c\ (mmol/mol) \div 10.929] + 2.152$$

Calibration of the Capillarys 3 system was performed by performing 3 successive series of analyses using HbA1c Capillary Calibrators 1 and 2 (Sebia, PN 4755). Although specific concentrations varied by lot, typical concentrations (%CV) for Calibrators 1 and 2 were 35 (5%) and 60 (7%) mmol/mol. Calibrators were shipped in a lyophilized form and were reconstituted with distilled water according to package instructions before use. The final HbA1c concentrations (H6HBA1C) ranged from 4.0 to 15.7% (20.19 - 148.07 mmol/mol).

4.2.3 Internal Quality Control

The Sebia system was maintained daily by cleaning machine components, replacing all reagents, and running Multi-system HbA1c Capillary Controls included in the kit (Sebia, PN 4768). Standard practice was to analyze the controls at the beginning and end of each analysis series, alternating between the two control samples.

5. External Quality Control

5.1 Reliability

Within a race/ethnicity- and sex-stratified random sample of 123 Add Health participants (89% fasting ≥ 8 hours) among whom venous blood was collected twice, on average 13.2 (95% confidence interval: 12.0–14.4) days apart, typically by the same FE and at approximately the same time of day, the reliability of glucose (mg/dl) and HbA1c (%) was estimated as an intra-class correlation coefficient (ICC, 95% confidence interval) [**Figure 6**]. The estimates suggest that the home exam venous blood collected at Add Health Wave VI yields a comparably reliable measure of HbA1c and a slightly less reliable measure of glucose than at Wave V.

Measure	N	ICC	95% CI
Glucose (mg/dl)	123	0.85	(0.80, 0.90)
HbA1c (%)	123	0.99	(0.98, 0.99)

Figure 6. Reliability of Glucose and HbA1c

6. Constructed Measures

6.1 Fasting Status [H6FASTTM]

During the home exam before blood was collected, all participants were asked the following question: "At what time did you last eat or drink anything other than water, including sugar-containing candy or gum?" Responses to the question were recorded in hours and minutes and designated AM or PM. All participants were then asked, "Was that today or yesterday?" To calculate fasting times, responses to the above question were subtracted from the tablet start-of-blood collection date/time stamp. Data on fasting times outside the 0-23.9-hour range were recoded to missing among 4 participants. Values that were deemed implausible outside of 0-23.9 were assigned a code of -9999.

6.2 Fasted for Eight Hours or More [H6FASTGL]

Participants who were fasting (≥ 8 hours) and non-fasting (< 8 hours) at the time of blood collection were identified (1,0).

6.3 Classification of Fasting Glucose [H6CFGLU]

The classification of glucose concentrations among Add Health participants who were fasting (≥ 8 hours) at the time of blood collection was constructed based on the 2020 American Diabetes Association clinical practice recommendations for the diagnosis and classification of diabetes.[3] Three classes of fasting glucose are defined in **Figure 7**:

Classification	Fasting Glucose (mg/dl)	ADA Class
1	≤ 99	Normal
2	100 - 125	Impaired Fasting Glucose (IFG) / Pre-Diabetes
3	≥ 126	Diabetes

Figure 7. Classification of Fasting Glucose

6.4 Classification of Non-Fasting Glucose [H6CNFGLU]

The classification of glucose concentrations among Add Health participants who were non- fasting (< 8 hours) at the time of blood collection was constructed based on the 2025 American Diabetes Association clinical practice recommendations for the diagnosis and classification of diabetes.[3] Two classes of non-fasting glucose are defined in **Figure 8**:

Classification	Non-Fasting Glucose (mg/dl)	ADA Class
1	< 200	Normal
2	≥ 200	Diabetes

Figure 8. Classification of Non-Fasting Glucose

6.5 Classification of HbA1c [H6CHBA1C]

The classification of HbA1c among Add Health participants was constructed without regard to fasting status at the time of blood collection based on the 2025 American Diabetes Association clinical practice recommendations for the diagnosis and classification of diabetes.[3] Three classes of HbA1c are defined in **Figure 9**:

Classification	HbA1c (%)	ADA Class
1	< 5.7	Normal
2	5.7 - 6.4	Pre-Diabetes
3	≥ 6.5	Diabetes

Figure 9. Classification of HbA1c

6.6 Anti-diabetic Medication Use [H6C_MED]

Use of a prescription medication in the past four weeks in one or more of the therapeutic classes defined elsewhere,[9] but also listed in **Figure 10** was assigned a value of 1. Non-use of a prescription medication in the past four weeks in one of the therapeutic classes listed below was assigned a value of 0.

Class	Label	Variable
358-099-***	Antidiabetic agents	H6C_MED
358-099-213	Sulfonylureas	
358-099-214	Non-sulfonylureas	
358-099-215	Insulin	
358-099-216	Alpha-glucosidase inhibitors	
358-099-271	Thiazolidinediones	
358-099-282	Meglitinides	
358-099-309	Miscellaneous antidiabetic agents	
358-099-314	Antidiabetic combinations	
358-099-371	Dipeptidyl peptidase 4 inhibitors	
358-099-372	Amylin analogs	
358-099-373	Incretin mimetics	
358-099-458	SGLT-2 inhibitors	

Figure 10. Anti-diabetic Medications

Therapeutically classified use of prescription medication in particular classes may confound biomarker-based estimates of disease prevalence or risk. For example, use of antidiabetic medications may confound glucose or HbA1c-based estimates of diabetes prevalence or cardiovascular disease risk. However, the (1,0) classifications should be used cautiously in the investigation or control of potential confounding, because selection biases often threaten the study of non-randomized medication exposures. [10,11]

6.7 Joint Classification of Diabetes [H6DIABJC]

Participants were flagged as having evidence of diabetes if they met **at least one** of the following criteria in **Figure 11**:

Criterion (≥ 1 of the following must be true)	Variable & Value
Fasting glucose ≥ 126 mg/dl	H6CFGLU = 3
Non-fasting glucose ≥ 200 mg/dl	H6CNFGLU = 2
HbA1c ≥ 6.5%	H6CHBA1C = 3
Self-reported history of diabetes except during pregnancy	H6CQ47B = 1
Anti-diabetic medication-use in the past 4 weeks	H6C_MED = 1

Figure 11. Criteria Used to Identify Diabetes

7. The Glucose Homeostasis Data File (bglua1c6.sas7bdat)

7.1. Structure

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

7.2. Contents

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

Variable Name	Variable Description
AID	Participant Identifier
H6GLUCOS	Glucose (mg/dl)
H6HBA1C	Hemoglobin A1c (%)
H6FASTTM	Hours since last ate
H6FASTGL	Fasted for 8+ hours
H6CFGLU	Classification of fasting glucose (ADA)
H6CNFGLU	Classification of non-fasting glucose (ADA)
H6CHBA1C	Classification of HbA1c (ADA)
H6CQ47B	CQ47b Ever Diagnosed with high blood sugar or diabetes
H6C_MED	Antidiabetic medication use
H6DIABJC	Diabetes joint classification

7.3. Use

Despite attempts to harmonize methods across Waves IV , V, and VI, important inter-Wave differences in protocols, biospecimens, assays, and data quality exist, as grey-highlighted here and above. Their existence suggests that the measures of glucose homeostasis described in Sections 1-6 may not be readily comparable from wave to wave. Caution should therefore be exercised when leveraging repeated measures of glucose homeostasis from Wave IV-VI, whether they are primary measures or constructed classifications. Indeed, the merit of pre-analytical z-transformation or quantile-based classification of Wave IV-VI biomarkers (glucose; HbA1c) and the potential pitfall otherwise associated with equating values in their original units (mg/dl; %) across visits should be carefully considered before using these data.

8. References

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