2025

Add Health Wave VI Documentation

Add Health

The National Longitudinal Study of Adolescent to Adult Health

Report prepared by

Robert A. Angel

Lixin Qu

Jason Grago

Kathryn S. Carrier

Robert A. Hummer

Eric A. Whitsel

Hepatic Injury User Guide

UNC CAROLINA POPULATION CENTER

CAROLINA POPULATION CENTER | CAROLINA SQUARE - SUITE 210 | 123 WEST FRANKLIN STREET | CHAPEL HILL, NC 27516

https://doi.org/10.17615/e84h-6t47

This document summarizes the rationale, equipment, measurement, protocol and data cleaning procedures for each of the hepatic measures collected at the Wave VI home exam. It also documents how constructed variables were derived from the hepatic measures collected in the field. 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
- Glucose Homeostasis
- Home Exam – Medication Use
- Home Exam Questionnaire and QC Metrics
- Infection
- Inflammation and Immune Function
- Lipids
- Neurodegeneration
- Renal Function

Acknowledgement

Data for Wave VI of Add Health were supported by two cooperative agreements from the National Institute on Aging (1U01AG071448, principal investigator Robert A. Hummer, and 1U01AG071450, principal investigators Robert A. Hummer and Allison E. Aiello) to the University of North Carolina at Chapel Hill. Co-funding for Wave VI was provided by the Eunice Kennedy Shriver National Institute of Child Health and Human Development, the National Institute on Minority Health and Health Disparities, the National Institute on Drug Abuse, the NIH Office of Behavioral and Social Science Research, and the NIH Office of Disease Prevention. Data from Waves I-V of Add Health are from the Add Health Program Project, grant P01 HD31921 (Kathleen Mullan Harris) from the Eunice Kennedy Shriver National Institute of Child Health and Human Development, with cooperative funding from 23 other federal agencies and foundations. Add Health was originally designed by J. Richard Udry, Peter S. Bearman, and Kathleen Mullan Harris at the University of North Carolina at Chapel Hill. Add Health is currently directed by Robert A. Hummer; it was previously directed by Kathleen Mullan Harris (2004-2021) and J. Richard Udry (1994- 2004). Information on obtaining Add Health data is available on the project website (https://addhealth.cpc.unc.edu).

Suggested Citation

Angel RA, Grago J, Qu L, Carrier KS, Hummer RA, Whitsel EA. Add Health Wave VI Documentation: Hepatic Injury, 2025; Available from: https://doi.org/10.17615/e84h-6t47

Table of Contents

1. Introduction

Assays of hepatic biomarkers at Wave V[1] 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 archiving. Samples were thawed at a later date and assayed for the following hepatic biomarkers:

Assayed Hepatic Biomarkers

- Aspartate Aminotransferase [AST] (U/L)
- Alanine Aminotransferase [ALT] (U/L)

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

- AST/ALT ratio
- Classification of the AST/ALT ratio

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 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 biomarkers of metabolic, hepatic, renal, amyloid-tau-neurodegenerative (ATN), inflammatory, immune, and infectious conditions, including the measures of hepatic injury 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 it, 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 the 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 tube 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.

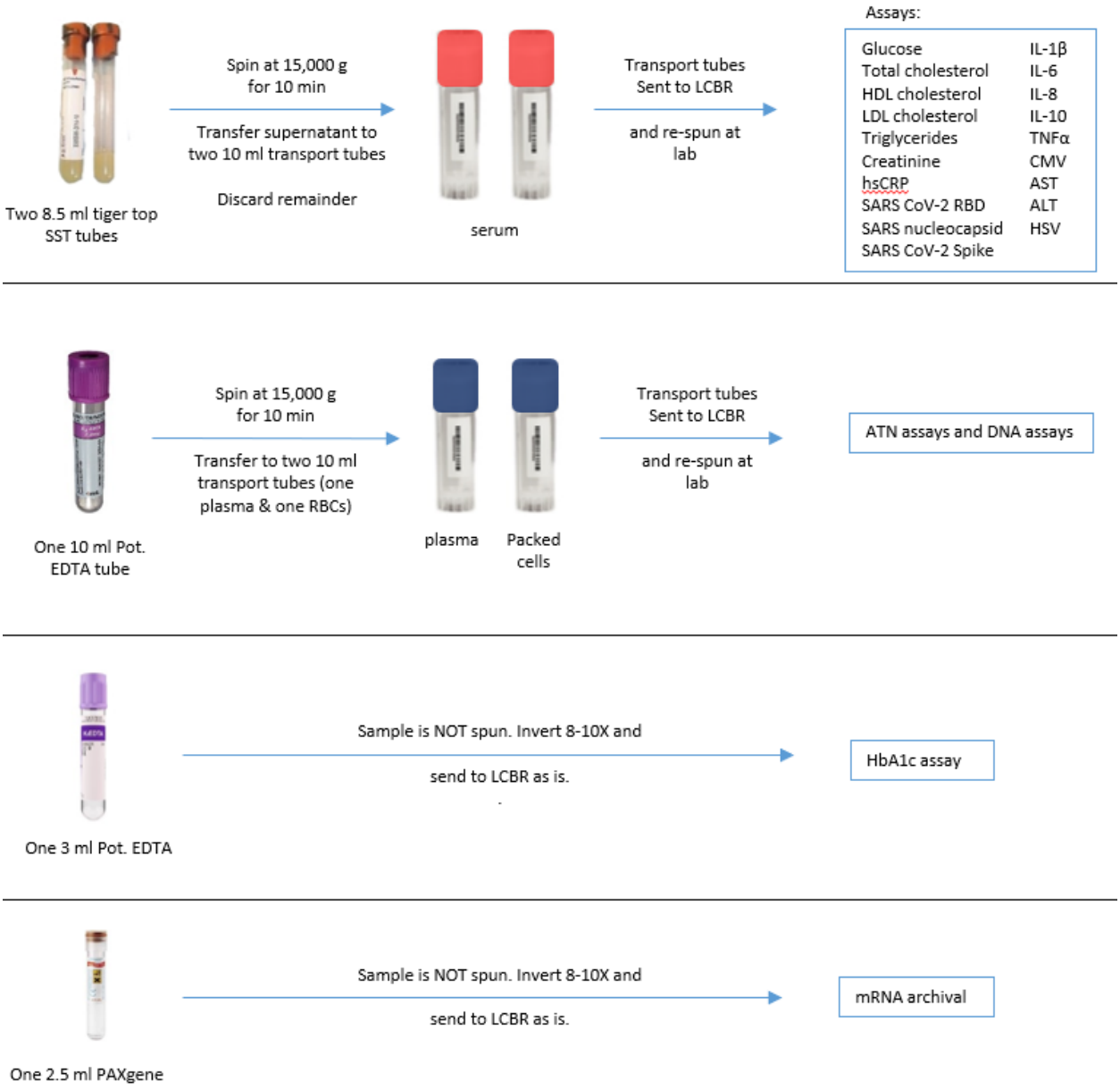

Figure 3. Blood Processing Protocol

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 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 (one 500 ul aliquot). The LCBR technicians entered all aliquot information into the LIMS system.

4. Assay and Internal Quality Control

4.1 Aspartate Aminotransferase, AST [H6AST]

4.1.1 Rationale

AST (formerly, serum glutamic-oxaloacetic transaminase [SGOT]) is an enzyme involved in amino acid metabolism that catalyzes the reversible transfer of an amino group between aspartate and glutamate. It is found, in decreasing order of concentration, in the liver, heart muscle, skeletal muscle, kidneys,

brain, pancreas, lungs, leukocytes, and red blood cells, so it can be used to help identify diseases involving them. Increases in AST are not specific to the liver, but they are common among diseases associated with extensive hepatocellular injury including viral, ischemic, or toxic (e.g. acetaminophen-related) hepatitis; non-alcoholic fatty liver disease; alcoholic liver disease; heart failure; and hepatic metastasis. Decreases can be found in patients undergoing renal dialysis or persons with vitamin B_6 deficiency.

4.1.2 Assay Protocol

All AST 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 AST 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 AST, other assays were run from the same serum sample, including ALT, glucose, total cholesterol, high-density lipoprotein cholesterol, triglycerides, and creatinine. This section describes the AST assay. Section 4.2 describes the ALT assay. Assay protocols for other analytes can be found in other Add Health User Guides.

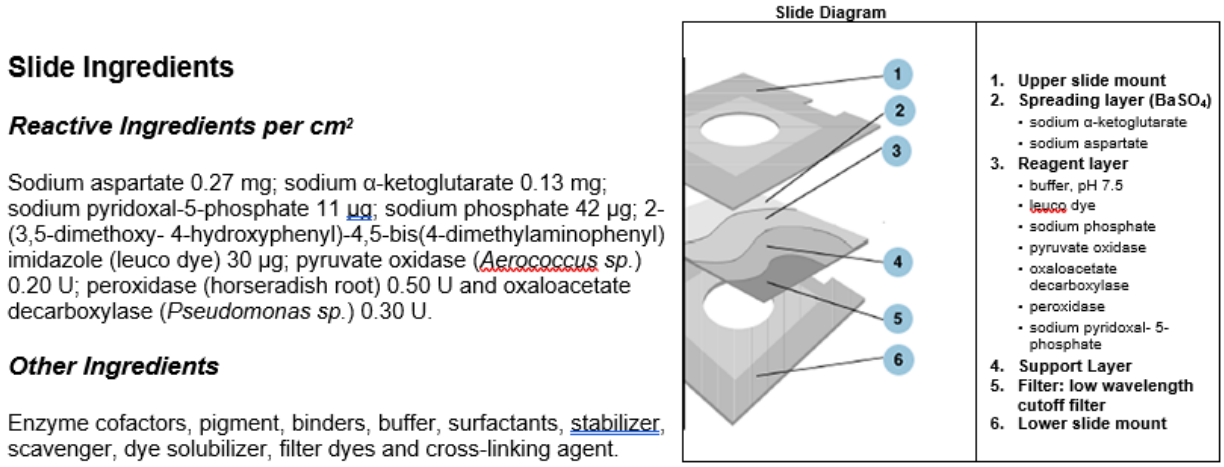

Figure 4. Ortho-Vitros AST Slide

Upon introduction of each vial into the analyzer, 10 µl of serum was aspirated, deposited onto an AST slide for analysis, and was evenly distributed by the spreading layer to the underlying layers. In the assay for AST, the amino group of L-aspartate was transferred to α-ketoglutarate in the presence of pyridoxal-5-phosphate (P-5-P) to produce glutamate and oxaloacetate. The oxaloacetate formed in the

deamination of the L-aspartate was converted to pyruvate and carbon dioxide by oxaloacetate decarboxylase. Pyruvate was oxidized to acetylphosphate and hydrogen peroxide by pyruvate oxidase. The final reaction step involved the peroxidase-catalyzed oxidation of a leuco dye to produce a colored dye. The rate of oxidation of the leuco dye was monitored by reflectance spectrophotometry. The rate of change in reflectance density was proportional to enzyme activity in the sample. The low wavelength cutoff filter on the slide support minimized the blank rate effects of incident light during the dye development. The specific reaction scheme is displayed in **Figure 5**.[2]

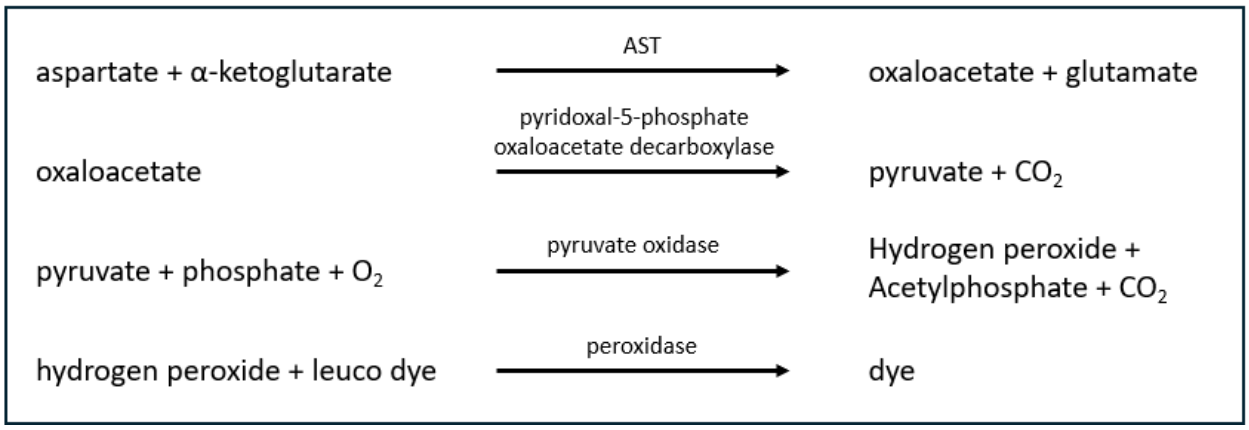

Figure 5. AST Assay Reaction Scheme

Based on sequential readings of the slide's reflectance at 670 nm over the defined incubation period, a rate of change in reflectance was determined. This rate was used in the software-resident multi-point rate calibration model to compute enzyme activity. Once a calibration was performed for each slide lot, AST activity in unknown samples was determined from the rate of change in reflectance measured for each unknown test slide. 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 AST assay was 3-750 U/L. 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 AST concentrations that accounted for the reflexive dilutions via multiplication by the dilution factor were reported simultaneously. The final AST concentrations (H6AST) ranged from 13 to 373 U/L.

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 AST, the low, middle, and high control values typically ranged from 30-50, 120-200, and 250-350 U/L respectively.

Values assigned to the VITROS Chemistry Products Calibrator Kit for AST are traceable to the AST method recommended by the International Federation of Clinical Chemistry (IFCC),[3] adapted to a centrifugal analyzer at 37 °C.

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 AST concentration mean (coefficient of variation) based on those assays was 24.2 U/L (4.1%) and 28.9 U/L (4.1%), respectively. When AST concentrations exceeded acceptable parameters, the Roche analyzer was investigated and repaired.

4.2 Alanine Aminotransferase, ALT [H6ALT]

4.2.1 Rationale

ALT (formerly serum glutamic-pyruvic transaminase [SGPT]) is an enzyme involved in amino acid metabolism that catalyzes the reversible transfer of an amino group from alanine to ketoglutarate yielding pyruvate and glutamate. It is found in many tissues, but at highest concentration in the liver, such that increases in ALT are more specific than AST for liver diseases like those listed in Section 4.1.1.

4.2.2 Assay Protocol

All ALT 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 ALT slides, i.e. multilayered, analytical elements coated on polyester supports (**Figure 6**). 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.

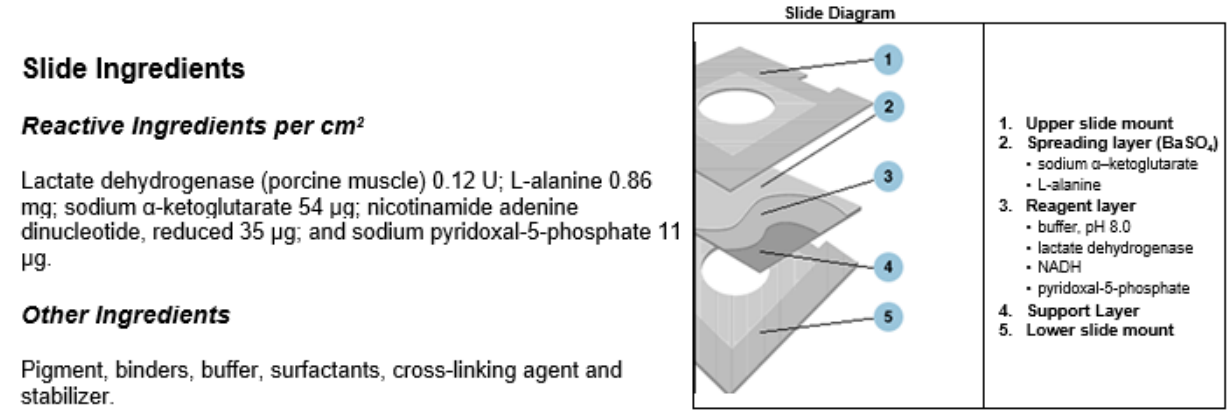

Figure 6. Ortho-Vitros ALT Slide

Upon introduction of each vial into the analyzer, 10 µl of serum was aspirated, deposited onto an ALT slide for analysis, and was evenly distributed by the spreading layer to the underlying layers. The spreading layer contained the ALT substrates L-alanine and sodium α-ketoglutarate. Alanine aminotransferase catalyzed the transfer of the amino group of L-alanine to α-ketoglutarate to produce pyruvate and glutamate. Lactate dehydrogenase (LDH) then catalyzed the conversion of pyruvate and NADH to lactate and NAD^+. The rate of oxidation of NADH was monitored by reflectance spectrophotometry. The rate of change in reflection density was proportional to enzyme activity. The specific reaction scheme is displayed in **Figure 7**.[4]

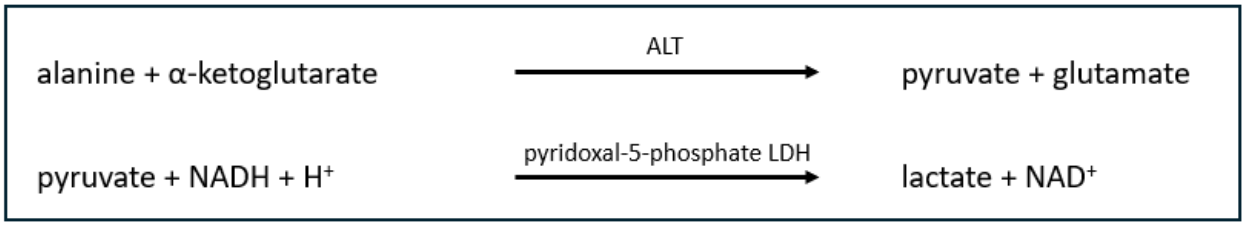

Figure 7. ALT Assay Reaction Scheme

The intensity of the dye was measured and corresponding ALT concentration inferred at room temperature by comparing reflected light output at 340 nm to a standard curve generated using a VITROS Chemistry Products Calibrator Kit (Ortho Clinical Diagnostics, Raritan, NJ). 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 ALT assay was 6-1000 U/L. 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 ALT concentrations that accounted for the reflexive dilutions via multiplication by the dilution factor were reported simultaneously. The final ALT concentrations (H6ALT) ranged from 5 to 631 U/L.

4.2.3 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 ALT, the low, middle, and high control values typically ranged from 25-45, 80-170, and 150-300 U/L respectively.

Values assigned to the VITROS Chemistry Products Calibrator Kit 3 for ALT are traceable to the ALT method recommended by the International Federation of Clinical Chemistry (IFCC),[5] adapted to a centrifugal analyzer at 37 °C.

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 V. The plasma and serum ALT concentration mean (coefficient of variation) based on those assays was 11.6 U/L (11.6%) and 15.16 U/L (7.5%), respectively. When ALT concentrations exceeded acceptable parameters, the Roche analyzer was investigated and repaired.

4.3 Serum Indices

4.3.1 Rationale

Endogenous and exogenous constituents in the sample matrix can interfere with laboratory assays. A colored appearance of samples or additional information can lead to their pre-analytical recognition. Hemolysis, icterus (bilirubin), and lipemia (turbidity), also can be detected analytically. Although interference by them is difficult to predict because of their strong method-dependence[6], three semi-quantitative serum index assays were performed to evaluate the possibility of interference with the AST or ALT assays.

4.3.2 Assay Protocol

All serum index assays were performed using the VITROS 5600 system that included a built-in VITROS MicroSensor™ Technology feature which could perform automated semi-quantitative sample quality index measurements. These sample quality indices measured the degree of hemolysis, icterus and Lipemia (turbidity) of a sample in a CuvaTip using a spectrophotometer. These assays were run in

tandem with the AST/ALT assays, incorporated as one procedure by the VITROS system. Default thresholds for hemolysis interference flags were set based on interference testing during system development and were specific for each assay.

The VITROS™ MicroSensor contains a solid-state micro-spectrophotometer, which utilized a diffraction grating and a 256-element photodiode array. The module provided energy spectra for samples to the master computer for index calculations. Measurements using the MircoSensor™ Technology had no impact on any system functions and did not consume any additional sample volume. **Figure 8** shows the MicroSensor™ process.

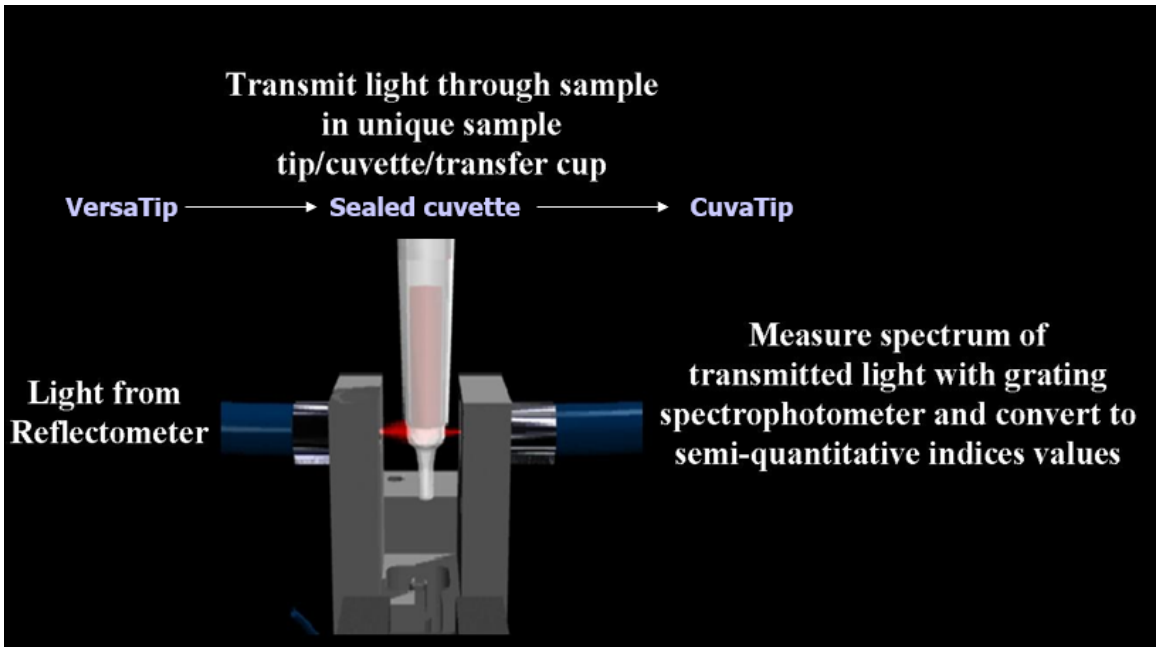

Figure 8. VITROS MicroSensor Technology Process

Hemolysis and Icterus calculations were based on the first derivative of defined absorbance spectrum:

$$H = [dA/d\lambda] * [a_H]$$

$$I = [dA/d\lambda] * [a_I]$$

where:
H = Hemolysis index
I = Icterus index
[dA/dλ] = Derivative absorbance vector
$[a_H]$ = Hemolysis coefficient vector (522-750 nM)
$[a_I]$ = Icterus coefficient vector (500-770 nM)

Turbidity calculations were calculated using absorbance at 700 nM:

$$T = \exp (a_3 A_{700}^3 + a_2 A_{700}^2 + a_0)$$

where:
T = Turbidity index
A_{700} = Absorbance at 700 nM
a_0, a_1, a_2, a_3 = Polynomial coefficients
Exp () = Exponential function e^x

Any aliquots that returned index values that were higher than the predetermined flags for hemolysis, icterus, or turbidity for either AST or ALT were reprocessed by UVMMC and then run again for the required assay. **Figure 9** shows the thresholds for the hemolysis index levels for both AST and ALT assays.

Assay	Hemolysis Index	Result
ALT	≥500	Sample rejected
AST	50-129	Sample accepted, slight hemolysis noted
AST	130-199	Sample accepted, moderate hemolysis noted
AST	≥200	Sample rejected

Figure 9. Hemolysis Levels for Serum Index Assays

Following a rerun, any ALT aliquot with a hemolysis index ≥ 500 or AST aliquot with a hemolysis index ≥ 200 was not reported. A total of 47 (1% of) AST aliquots had a hemolysis index of 50-200 and were reported as "*poor quality – result reported*" (see section 6.3). No aliquots had elevated icterus or lipemia indices.

5. External Quality Control

5.1 Reliability

Within a race/ethnicity- and sex-stratified random sample of 123 Add Health participants 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 AST (U/L) and ALT (U/L) was estimated as an intra-class correlation coefficient (ICC, 95% confidence interval) [**Figure 10**]. The estimates suggest that home exam venous blood collected at Add Health Wave VI yields a slightly more reliable measure of ALT than AST, as it did at Wave V.

Measure	N	ICC	95% CI
AST (U/L)	123	0.76	(0.68, 0.83)
ALT (U/L)	123	0.85	(0.80, 0.90)

Figure 10. Reliability of AST and ALT

6. Constructed Measures

6.1 AST to ALT ratio [H6ASTALT]

While hepatic assays are often insufficiently specific to allow definitive diagnosis of liver disease, a ratio of AST to ALT concentrations > 2 is nonetheless suggestive of alcoholic liver disease.[7] The AST to ALT concentration ratio was therefore calculated by dividing AST (U/L) by ALT (U/L), as follows:

$$\text{H6ASTALT} = \frac{\text{AST (U/L)}}{\text{ALT (U/L)}}$$

6.2. Classification of AST to ALT ratio [H6CRATIO]

The AST to ALT ratio was then classified accordingly (**Figure 11**).

Classification	AST to ALT ratio	Description
1	≤ 2	Not suggestive of alcoholic liver disease
2	> 2	Suggestive of alcoholic liver disease

Figure 11. Classification of AST to ALT Ratio

6.3 Flag indicating poor quality AST samples [H6ASTFL]

As stated in section 4.3.2, any AST samples with a hemolysis index of 50-200 were reported but flagged as poor quality, shown in **Figure 12**.

Code	Description
1	AST result normal
2	AST result with poor quality

Figure 12. Flag Indicating Quality of AST results

7. The Hepatic Data File (bhepat6.sas7bdat)

7.1. Structure

The structure of the disseminated hepatic 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 hepatic 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
H6AST	Aspartate aminotransferase (AST, U/L)
H6ALT	Alanine aminotransferase (ALT, U/L)
H6ASTALT	AST/ALT ratio
H6CRATIO	Classification of AST/ALT ratio
H6ASTFL	AST results quality

7.3. Use

Despite attempts to harmonize methods across Waves 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 hepatic injury described in Sections 1-6 may not be readily comparable from wave to wave. Caution should therefore be exercised when leveraging repeated measures of hepatic injury from Wave V-VI, whether they are primary measures or constructed classifications. Indeed, the merit of pre-analytical z-transformation or quantile-based classification of Wave V-VI biomarkers (AST; ALT) and the potential pitfall otherwise associated with equating values in their original units (U/L) across visits should be carefully considered before using these data.

8. References

1. Whitsel EA, Angel R, O'Hara R, Qu L, Carrier K, Harris K. *Add Health Wave V Documentation: Hepatic Injury*, 2020; Available from: https://doi.org/10.17615/m1xd-rm12.
2. Ortho-Clinical Diagnostics, *VITROS Chemistry Products AST slides Instructions for Use*, Version 13.0, Pub. No. MP2-113_EN, Rochester, NY, 2015.
3. Bergmeyer HU, Hørder M, Rej R. Approved Recommendation (1985) on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes. Part 2. IFCC Method for Aspartate Aminotransferase. *J Clin Chem Clin Biochem* 1986;24(7):497-510.
4. Ortho-Clinical Diagnostics, *VITROS Chemistry Products ALT slides Instructions for Use*, Version 11.0, Pub. No. MP2-36_EN, Rochester, NY, 2015.
5. Bergmeyer HU, Horder M, Rej R. Approved Recommendation (1985) on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes. Part 3. IFCC Method for Alanine Aminotransferase. *J Clin Chem Clin Biochem* 1986;24(7):481-495.
6. Guder WG, da Fonseca-Wolheim F, Heil W, Schmitt YM, Töpfer G, Wisser H, Zawta B. The Haemolytic, Icteric and Lipemic Sample Recommendations Regarding their Recognition and Prevention of Clinically Relevant Interferences. Recommendations of the Working Group on Preanalytical Variables of the German Society for Clinical Chemistry and the German Society for Laboratory Medicine. *J Lab Med* 2000;24(8):357-364.
7. Cohen JA, Kaplan MM. The SGOT/SGPT Ratio—An Indicator of Alcoholic Liver Disease. *Dig Dis Sci* 1979;24(11):835-838.