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# Hepatic Injury



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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.

During Wave V, measures of biomarkers were obtained to best mirror those collected in Wave IV. Their protocols and descriptions are documented in the following series of user guides available on the Add Health website.

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

Additional assays were subsequently performed using archived blood samples from Wave V to mirror those measures that will be analyzed in Add Health Wave VI. None of these additional assays were performed during Wave IV. This document summarizes the rationale, equipment, protocol, assays, internal quality control, data cleaning, external quality control, and procedures for the measurement and classification of hepatic injury using Wave V blood samples. It is the first in a set of additional Wave V user guides:

- Hepatic Injury
- Neurodegeneration
- Additional Measures of Inflammation and Immune Function

## Acknowledgement

This research uses data from Add Health, funded by grant P01 HD31921 (Harris) from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), with cooperative funding from 23 other federal agencies and foundations. Add Health is currently directed by Robert A. Hummer and funded by the National Institute on Aging cooperative agreements U01 AG071448 (Hummer) and U01AG071450 (Aiello and Hummer) at the University of North Carolina at Chapel Hill. Add Health was designed by J. Richard Udry, Peter S. Bearman, and Kathleen Mullan Harris at the University of North Carolina at Chapel Hill.

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## 1. Introduction

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)
- Lipemia Index [H5L\_INDX]
- Hemolysis Index [H5H\_INDX]
- Icterus Index [H5I\_INDX]

Moreover, the restricted use Add Health Wave V data includes two constructed measures:

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

## 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.

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 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

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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. It also was collected to establish an archive of serum, plasma, whole blood, RNA, and packed cells capable of supporting future ancillary studies and assays including those of the hepatic biomarkers described herein.

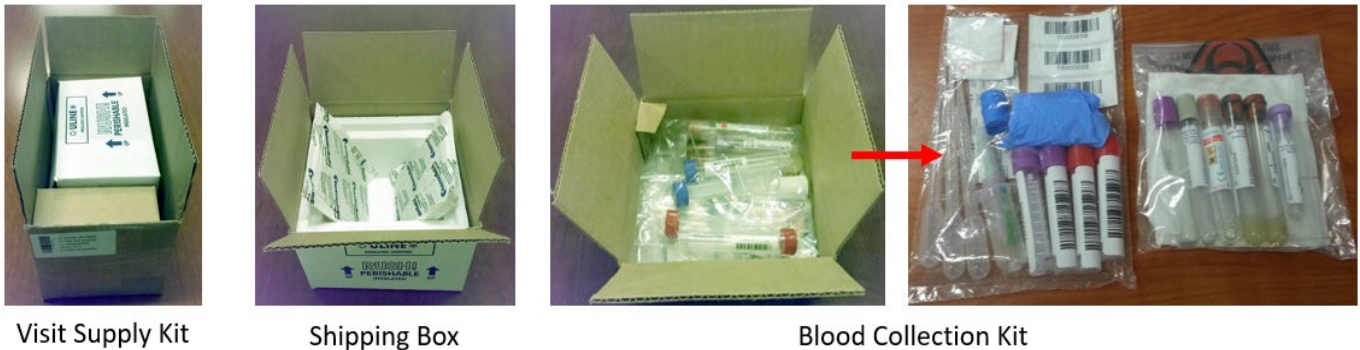
### 3.2 Equipment

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 Glucose Homeostasis User Guide, 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 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.

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- 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.
- Quickly push the first vacutainer tube (using the sequence in the table above) 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).



## Exhibit 2. Tubes of Blood Collected

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	Assays: DNA 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 NaFI/KOx	Yes	Plasma	Discarded	Assay: glucose sub-study
6	10 ml PAXgene	No	N/A	N/A	Archival: for future use

\*Also included aspartate aminotransferase, alanine aminotransferase, and serum indices, as described below

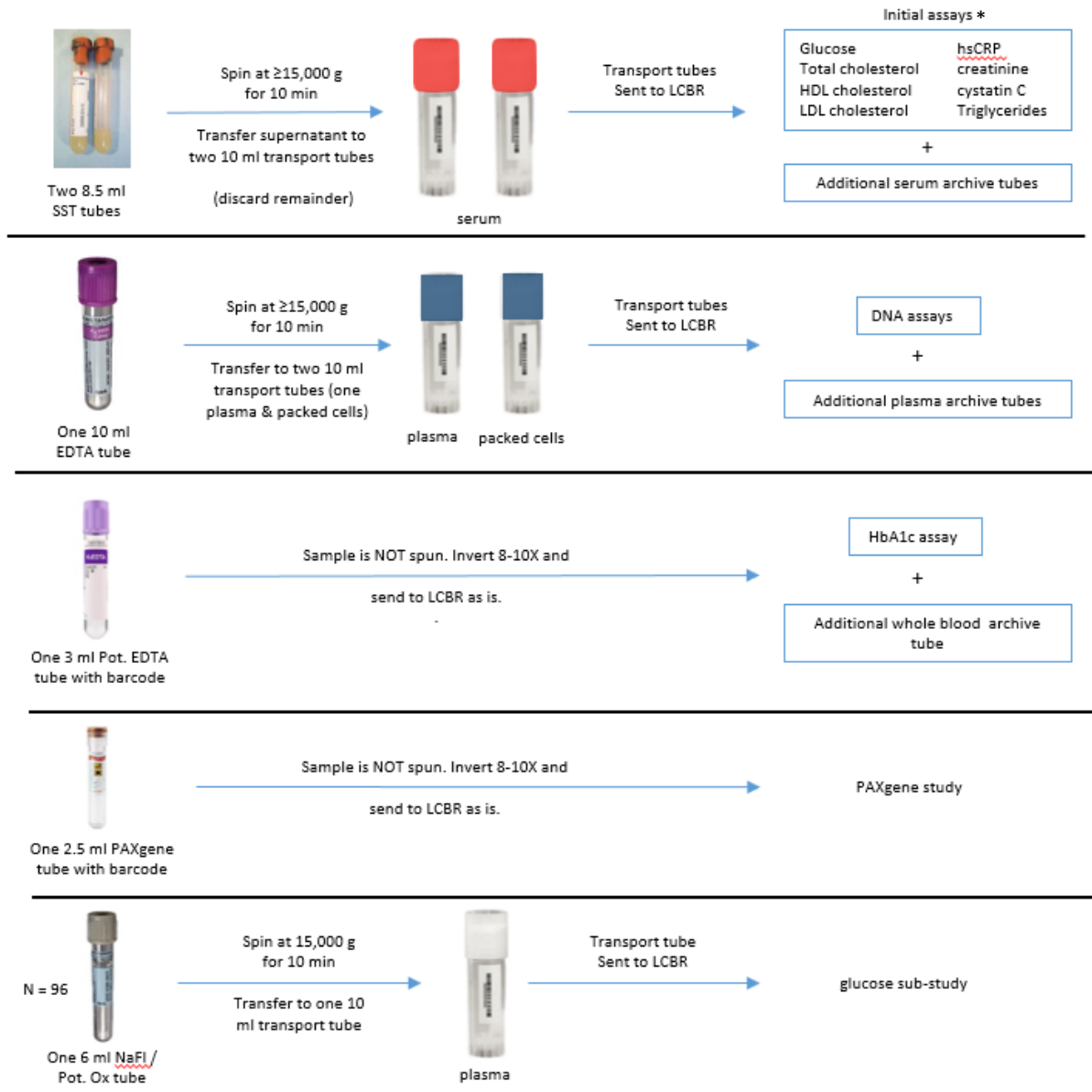
### 3.3.2 Blood Processing

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 NaFI/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 NaFI/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/FI/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.

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### Exhibit 3. Blood Processing Protocol



\*Also included aspartate aminotransferase, alanine aminotransferase and serum indices, as described below

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 the

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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 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.

### 3.3.5 Preparation of Samples for Hepatic Assays

All hepatic assays were performed using archived serum samples stored originally at the time of initial processing. A 1 ml archive tube of serum from each respondent was selected, thawed in a 36°C water bath for 10 minutes, and re-vortexed on the day their sample was to be assayed. This tube was placed directly in the Roche assay machine (see below), and the required serum was withdrawn from it. Afterwards, the remainder of the sample was refrozen and re-archived at -80°C. All assays were performed within a range of 270-1312 days after the samples were initially archived (mean=706 days, SD=302 days).

## 4. Assay and Internal Quality Control

### 4.1 Aspartate Aminotransferase, AST [H5AST]

#### 4.1.1 Rationale

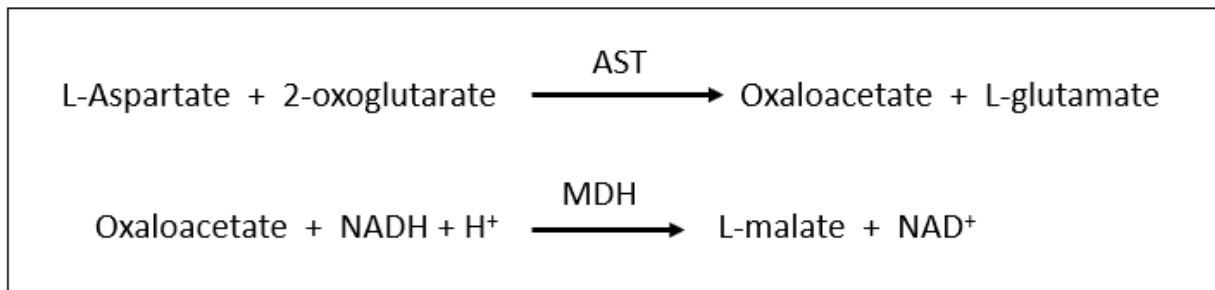
AST (formerly, serum glutamic-oxaloacetic transaminase [SGOT]) is an enzyme involved in amino acid metabolism that catalyzes the reversible transfer of an amino group between aspartate and glutamate. It is found, in decreasing order of concentration, in the liver, heart muscle, skeletal muscle, kidneys, 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<sub>6</sub> deficiency.

#### 4.1.2 Assay Protocol

All AST assays were performed using a Roche Cobas C311 analyzer (Roche Diagnostics, Indianapolis, IN) and a Cobas Aspartate Aminotransferase According to International Federation of Clinical Chemistry (IFCC) Without Pyridoxal Phosphate Activation assay. Serum from venous blood collected using the SST vacutainer tubes was introduced into the Roche system by placing thawed and re-vortexed sample vials of serum into an automatic sampling tray, after which all processes were automatically performed and results output by the Roche system. The Roche system was programmed to automatically run all hepatic assays. All assays were run using the same sample. Approximately 9 ul was used for the AST assay.

Once the sample was introduced into the analyzer, AST in the sample catalyzed the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacted with NADH, in the presence of malate dehydrogenase (MDH), to form NAD<sup>+</sup>.<sup>1</sup> The rate of the NADH oxidation was directly proportional to the catalytic AST activity. It was determined by measuring the decrease in absorbance at 700 nm (main) and 340 nm (sub). This assay followed the recommendations of the IFCC, but was optimized for performance and stability.<sup>2,3</sup> The assay reaction scheme is illustrated in Exhibit 4.

**Exhibit 4. AST Assay Reaction Scheme**



The Cobas C311's dynamic measuring range for this assay was 5-700 U/L. When concentrations exceeded the upper limit, the C311 employed a "rerun" function, which automatically diluted the samples 1:10 with 9% sodium chloride (NaCl) and then re-assayed them 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 concentration (H5AST) ranged from 5 to 380 U/L.

#### 4.1.3 Internal Quality Control

The Roche system was maintained by cleaning machine components, replacing all reagents, and running known quality control samples according to Roche specifications. Two-point calibration using water and an AST standard (105 U/L) provided in the assay kit was performed each time a new lot of reagents was used or if QC checks found the calibration to be out of defined limits. This method was standardized against the original IFCC formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity,  $\epsilon$ .<sup>4</sup>

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 AST concentration

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mean (coefficient of variation) based on those assays was 22 U/L (7.10%) and 24 U/L (4.48%), respectively. When AST concentrations exceeded acceptable parameters, the Roche analyzer was investigated and repaired.

## 4.2 Alanine Aminotransferase, ALT [H5ALT]

### 4.2.1 Rationale

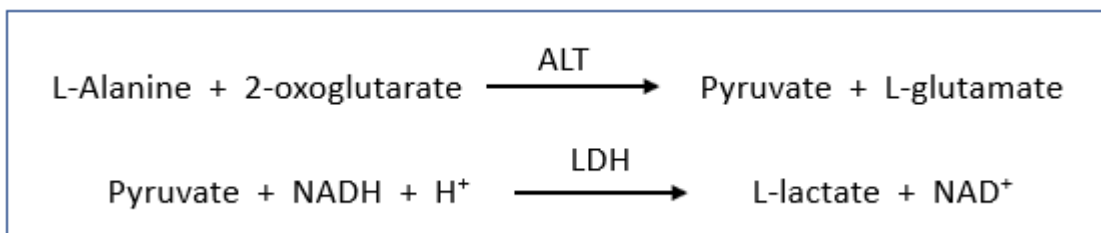
ALT (formerly serum glutamic-pyruvic transaminase [SGPT]) is an enzyme involved in amino acid metabolism that catalyzes the reversible transfer of an amino group from alanine to ketoglutarate yielding pyruvate and glutamate. It is found in many tissues, but at highest concentration in the liver, such 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 performed using a Roche Cobas C311 analyzer (Roche Diagnostics, Indianapolis, IN) and a Cobas *Alanine Aminotransferase according to IFCC Without Pyridoxal Phosphate Activation* assay. Serum from venous blood collected using the SST vacutainer tubes was introduced into the Roche system by placing thawed and re-vortexed sample vials of serum into an automatic sampling tray, after which all processes were automatically performed and results output by the Roche system. The Roche system was programmed to automatically run all hepatic assays. All assays were run using the same sample. Approximately 9 ul was used for the ALT assay.

Once the sample was introduced into the analyzer, ALT catalyzed the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed was reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD<sup>+</sup>.<sup>5</sup> The rate of the NADH oxidation was directly proportional to the catalytic ALT activity. It was determined by measuring the decrease in absorbance at 700 nm (main) and 340 nm (sub). This assay followed the recommendations of the IFCC, but was optimized for performance and stability.<sup>6,7</sup> The assay reaction scheme is illustrated in Exhibit 5.

#### Exhibit 5. ALT Reaction Scheme



The Cobas C311's dynamic measuring range for this assay was 5-700 U/L. When concentrations exceeded the upper limit, the C311 employed a "rerun" function, which automatically diluted the samples 1:10 with 9% NaCl and then re-assayed them 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 concentration (H5ALT) ranged from 5 to 303 U/L.

### 4.2.3 Internal Quality Control

The Roche system was maintained by cleaning machine components, replacing all reagents, and running known quality control samples according to the Roche specifications. Two-point calibration using water

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and an ALT standard (89.2 U/L) provided in the assay kit was performed each time a new lot of reagents was used or if QC checks found the calibration to be out of defined limits. This method was standardized against the original IFCC formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity,  $\epsilon$ .<sup>8</sup>

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 ALT concentration mean (coefficient of variation) based on those assays was 21 U/L (6.10%) and 22 U/L (3.26%), respectively. When ALT concentrations exceeded acceptable parameters, the Roche analyzer was investigated and repaired.

### 4.3 Serum Indices [H5L\_INDX, H5H\_INDX, H5I\_INDX]

#### 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. Lipemia (turbidity), hemolysis, and icterus (bilirubin) also can be detected analytically. Although interference by them is difficult to predict because of their strong method-dependence,<sup>9</sup> three semi-quantitative serum index assays were performed to evaluate the possibility of interference with the AST or ALT assays:

- a. Lipemia Index [H5L\_INDX]
- b. Hemolysis Index [H5H\_INDX]
- c. Icterus Index [H5I\_INDX]

#### 4.3.2 Assay Protocol

All Serum Index assays were performed using a Roche Cobas C311 analyzer (Roche Diagnostics, Indianapolis, IN) and a Cobas *Serum Index Gen.2* assay. Serum from venous blood collected using the SST vacutainer tubes was introduced into the Roche system by placing thawed and re-vortexed sample vials of serum into an automatic sampling tray, after which all processes were automatically performed and results output by the Roche system. The Roche system was programmed to automatically run all hepatic assays required. All assays were run using the same sample. Approximately 6  $\mu$ l was used for the Serum Index assays.

The *Serum Index Gen.2* assay was based on measured absorbance of diluted samples at different bichromatic wavelength pairs that provided semi-quantitative representations of lipemia, hemolysis and icterus present in the serum samples.<sup>10</sup> Once each sample was introduced, the Cobas system aspirated an aliquot of the serum and diluted it in saline (0.9 % NaCl) to measure the absorbances for lipemia at 660 nm (primary wavelength) and 700 nm (secondary wavelength), for hemolysis at 570 nm (primary wavelength) and 600 nm (secondary wavelength), and for icterus at 480 nm (primary wavelength) and 505 nm (secondary wavelength). The instrument used proprietary algorithms to calculate the serum indices from these unitless absorbance values.

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The Cobas C311's dynamic reporting range for the Serum Indices was 10-2000 for lipemia, 5-1200 for hemolysis, and 0.5-60 for icterus. Serum Index summary statistics and thresholds for possible interference are tabulated in Exhibit 6. The thresholds were used to identify possible AST and ALT assay interference as described in Section 6.3, below.

**Exhibit 6. Serum Indices for AST and ALT assays**

Index	N	Mean	Standard Deviation	AST Possible Interference	ALT Possible Interference
<b>Lipemia</b>	4814	33.83	56.09	> 150	> 150
<b>Hemolysis</b>	4814	5.90	13.37	> 40	> 90
<b>Icterus</b>	4814	0.74	0.52	> 60	> 60

#### 4.3.3 Internal Quality Control

The Roche system was maintained by cleaning machine components, replacing all reagents, and running known quality control samples according to the Roche specifications. Calibration was performed using water as a blank sample.

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 index mean (coefficient of variation) for the Lipemia Assay were 36.47 (7.20%) and 34.44 (9.5%) respectively. Means (coefficients of variation) for both the Hemolysis and Icterus assays were 0.0 (0.0%) in both plasma and serum samples.

## 5. External Quality Control

### 5.1 Reliability

Within a race/ethnicity- and sex-stratified random sample of 105 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 AST (U/L) and ALT (U/L) was estimated as an intra-class correlation coefficient (ICC, 95% confidence interval) [Exhibit 7]. The estimates suggest that the home exam venous blood collected at Add Health Wave V yields a more reliable measure of AST and ALT.

**Exhibit 7. Reliability of AST and ALT**

Measure	N	ICC	95% CI
<b>AST (U/L)</b>	105	0.74	(0.65, 0.83)
<b>ALT (U/L)</b>	105	0.90	(0.86, 0.94)

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## 6. Constructed Variables

### 6.1 AST to ALT ratio [H5ASTALT]

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.<sup>11</sup> The AST to ALT concentration ratio was therefore calculated by dividing AST (U/L) by ALT (U/L), as follows:

$$\mathbf{H5ASTALT} = \frac{\mathbf{AST (U/L)}}{\mathbf{ALT (U/L)}}$$

### 6.2. Classification of AST to ALT ratio [H5CRATIO]

The AST to ALT ratio was then classified accordingly (Exhibit 8).<sup>11</sup>

**Exhibit 8. Classification of AST to ALT Ratio**

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

### 6.3. Classification of Possible AST and ALT Assay Interference [H5ASTINT, H5ALTINT]

As introduced in Section 4.3, three semi-quantitative serum indices were assayed to evaluate the possibility of AST and/or ALT assay interference by lipemia, hemolysis and/or icterus. Because no value of the Icterus Index exceeded the threshold for possible interference, only values of the Lipemia and Hemolysis Indices were used to flag it, as tabulated below (Exhibits 9-10).

**Exhibit 9. AST Interference Status**

H5ASTINT Code	Possible AST Interference
0	None
1	Possible Lipemia Interference (H5L_INDX > 150)
2	Possible Hemolysis Interference (H5H_INDX > 40)
3	Possible Lipemia & Hemolysis Interference (H5L_INDX > 150 & H5H_INDX > 40)

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### Exhibit 10. ALT Interference Status

H5ALTINT Code	Possible ALT Interference
0	None
1	Possible Lipemia Interference (H5L_INDX > 150)
2	Possible Hemolysis Interference (H5H_INDX > 90)
3	Possible Lipemia & Hemolysis Interference (H5L_INDX > 150 & H5H_INDX > 90)

## 7. The Hepatic Data File (bhepat5.xpt)

### 7.1. Structure

The structure of the disseminated hepatic 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 hepatic data file includes the variables below, which are described in the corresponding codebook documentation that also contains frequencies.

<b><u>Variable Name</u></b>	<b><u>Variable Description</u></b>
AID	Respondent identifier
H5AST	Aspartate aminotransferase (U/L)
H5ALT	Alanine aminotransferase (U/L)
H5ASTALT	AST/ALT ratio
H5CRATIO	Classification of AST/ALT ratio
H5L_INDX	Lipemia Index
H5H_INDX	Hemolysis Index
H5I_INDX	Icterus Index
H5ASTINT	Possible AST Interference
H5ALTINT	Possible ALT Interference

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