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Add Health Wave VI Documentation

Add Health

The National Longitudinal Study of Adolescent to Adult Health

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Wave VI Neurodegeneration

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This document summarizes the rationale, equipment, protocol, assay, internal quality control, data cleaning, external quality control, and procedures for the measurement and classification of neurodegeneration 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 V-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
- Hepatic Injury
- Home Exam – Medication Use
- Home Exam Questionnaire and QC Metrics
- Infection
- Inflammation and Immune Function
- Lipids
- Renal Function

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1. Introduction

Wave VI measures of neurodegeneration were based on venous blood collected via phlebotomy. 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, sent overnight to a laboratory, archived at -80°C, subsequently thawed at 36°C, and then assayed.

Assayed Neurodegeneration Biomarker Concentrations

- Neurofilament Light (NfL, pg/ml)
- Total Tau (pg/ml)
- Glial Fibrillary Acidic Protein (GFAP, pg/ml)

Moreover, the restricted use Add Health Wave VI data included three constructed measures designed to facilitate analysis and interpretation of neurodegeneration biomarker concentrations:

- Flag Indicating the NfL Concentration Type
- Flag Indicating the Tau Concentration Type
- Flag Indicating the GFAP Concentration Type

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 pre-specified biomarkers of metabolic, hepatic, renal, amyloid-tau-neurodegenerative (ATN), inflammatory, immune, and infectious conditions, including the measures of neurodegeneration 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
- 10 ml EDTA-containing vacutainer tube
- 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 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, 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.

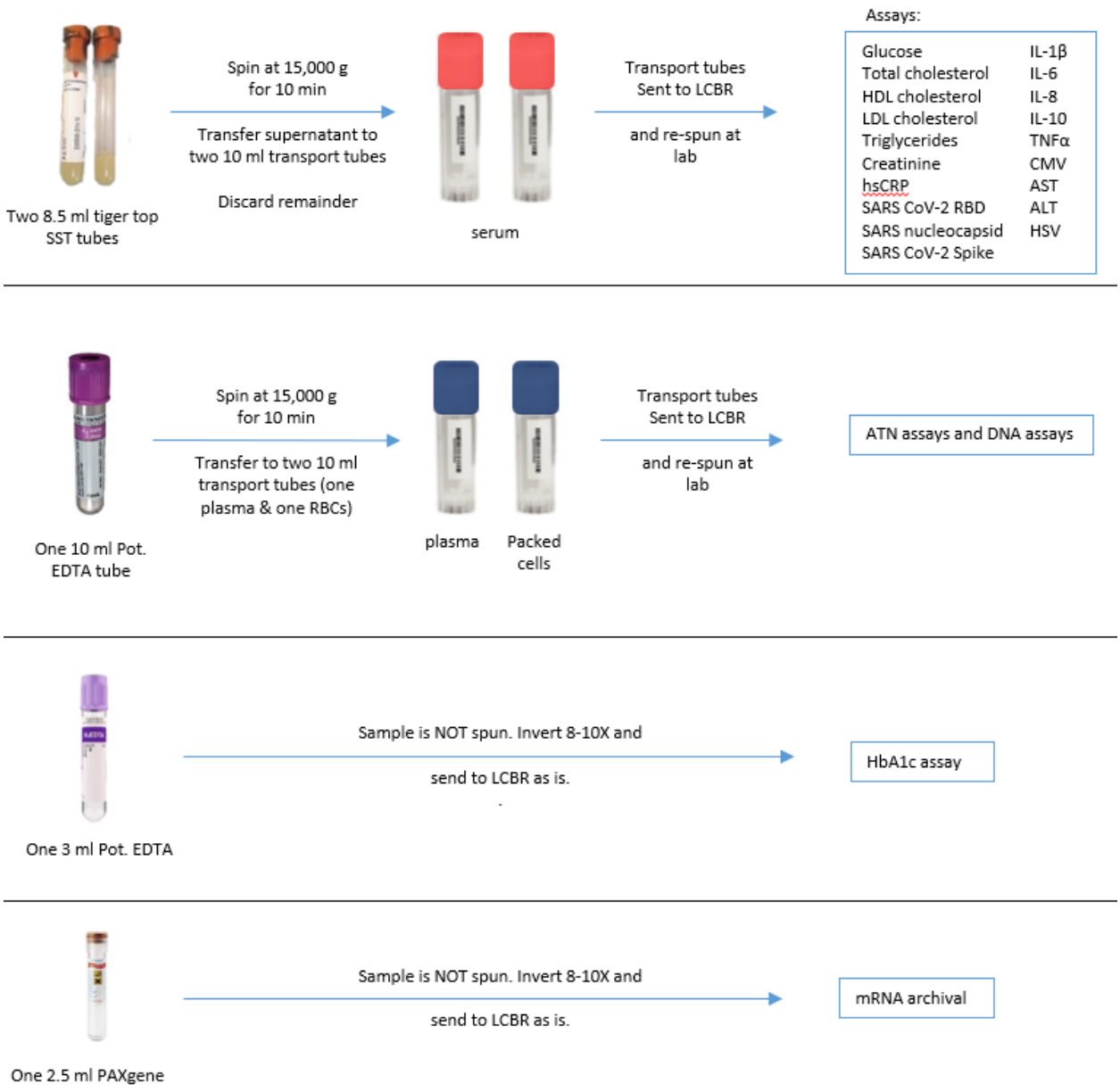

Figure 3. Blood Processing Protocol

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

3.3.5 Preparation of Samples for Neurodegeneration Biomarker Assays

The neurodegeneration assays were run using archived plasma samples. On the day of assay, 1 ml plasma aliquots initially archived at -80°C were thawed in a 36°C water bath for 10 minutes and vortexed and centrifuged. From the aliquots, 300 µl of plasma was sub-aliquoted and the remaining specimens re-archived at -80°C. Samples were run in batches, with 2466 samples run at the end of 2023, 2422 samples run at the end of 2024, and all remaining samples (n=623) run after the end of data collection in July 2025. On average, assays were performed approximately 6.5 months after the initial archival of the sample (mean = 199 days; standard deviation = 94 days; range = 7-517 days).

4. Assay and Internal Quality Control

4.1 NfL [H6NFL], Tau [H6TAU] and GFAP [H6GFAP]

4.1.1 Rationale

4.1.1.1 NfL [H6NFL]

Neurofilament light (NfL) is a cytoskeletal polypeptide that is expressed in neurons. It helps provide structural support for, regulate the diameter of, and control transmission of electrical impulses along the neuronal axon, thereby contributing to regular synaptic function.[1] Significant releases of NfL into both cerebrospinal fluid (CSF) and blood can follow axonal damage secondary to acute traumatic brain injury, stroke, Alzheimer's disease, frontotemporal dementia, amyotrophic lateral sclerosis, multiple sclerosis, and other neurological diseases.[2,3,4] Increases in it are therefore thought to reflect axonal damage and or degeneration. Until recently, NfL concentrations could only be measured in CSF, but new technologies such as the higher sensitivity Simoa kit used here also allow it to be measured in serum.

4.1.1.2 Tau [H6TAU]

Tau is a microtubule-stabilizing protein primarily localized in central nervous system neurons but also expressed at low levels in astrocytes and oligodendrocytes. CSF elevation of tau is observed in neurodegenerative disease and brain injury, suggesting its extracellular release during neuronal damage and role as a specific biomarker. Potential movement of elevated CSF tau across the blood-brain barrier also suggests that measurement of tau in peripherally sampled biospecimens, such as plasma, may provide a window into the levels of Tau accumulation in the brain.[1] The Simoa™ tau assay employed here uses a combination of monoclonal antibodies that react with both normal and phosphorylated tau. With an epitope in the mid-region of the molecule, the assay recognizes all six tau isoforms in the human brain (molecular weight range = 48,000-67,000 Da) and thus measures total tau.

4.1.1.3 GFAP [H6GFAP]

Glial Fibrillary Acidic Protein (GFAP) is a class-III intermediate filament majorly expressed in astrocytes in the central nervous system. Astrocytes play a variety of key roles in supporting, guiding, nurturing, and signaling neuronal architecture and activity. Monomeric GFAP is about 55kD. It is capable of forming both homodimers and heterodimers; GFAP can polymerize with other type III proteins or with neurofilament protein (such as NfL). GFAP is involved in many important central nervous system processes, including cell communication and the functioning of the blood brain barrier. GFAP also is associated with multiple neurological diseases and conditions such as traumatic brain injury, dementia, stroke, and brain tumors. Decreases in GFAP expression have been reported in Down's syndrome, schizophrenia, bipolar disorder, and depression.[1]

4.1.2 Assay Protocol

All plasma sample assays were performed at room temperature using a Single molecule array (Simoa™) HD-1 analyzer (Quanterix Corporation, Lexington, MA) and a 2-step, Quanterix multi-analyte Human Neurology 4-Plex A (N4PA) assay that provided quantitative determinations of Human NfL, Tau, and GFAP.

In this assay, antibody capture agents for the target analytes were attached to the surface of 2.7 µm paramagnetic beads included in the assay kit. Each capture agent-bead complex for the various analytes was marked with a fluorescent label of a different wavelength. 300 µl of plasma from the thawed 1 ml aliquot was diluted 1:4 using a sample diluent included in the kit. All other reagents including calibrator standards and controls were prepared according to manufacturer's recommendations. Samples, calibrators, and controls were added to the paramagnetic beads along with the presence of a biotinylated detection antibody. Target analyte molecules present in the sample were captured by the antibody coated beads and bound with the detection antibody simultaneously. The beads were then washed to remove non-specifically bound proteins and incubated with β-D-galactopyranoside (RGP)-labelled streptavidin.

After washing, the beads were then transferred to a Simoa image disc and individual beads were sealed within microwells in the array. The addition of β-galactosidase hydrolyzed the RGP substrate in the microwell into a fluorescent product that was then measured by the HD-1 analyzer. The protein concentration in the test sample was determined by counting the number of wells containing both a bead and fluorescent product relative to the total number of wells containing beads. The specific fluorescent labels on each type of capture agent-bead complex enabled the HD-1 analyzer to discriminate among analytes. Quantitative levels of all analytes were output simultaneously. Because Simoa enabled concentrations to be determined digitally rather than by measurement of the total analog signal, this approach to detecting single immunocomplexes has been termed a digital enzyme-linked immunosorbent assay (ELISA).[5] A typical assay workflow is illustrated in **Figure 4**.

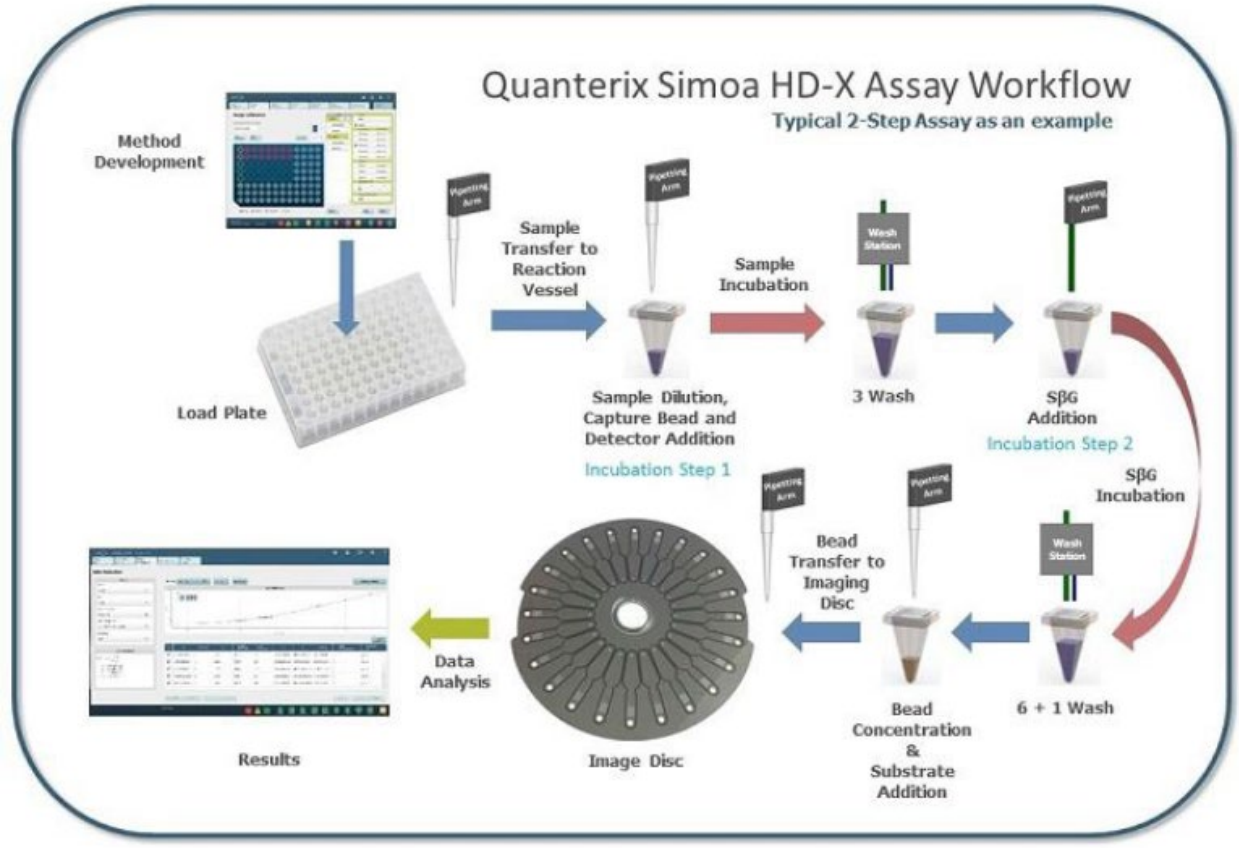

Figure 4. Simoa 2-Step Assay Protocol

4.1.3 Internal Quality Control

4.1.3.1 Simoa Assay Quality Control

According to manufacturer recommendations, the HD-1 system was maintained daily by checking reagent levels, waste levels, and cleaning machine components. It also underwent a monthly maintenance routine that included disposing/replacing all fluids in the machine, cleaning fluid paths, running a preloaded maintenance program, and using the Simoa™ Qualification Test assay to verify the sensitivity, precision, and accuracy of the HD-1 system.[5]

For each batch of n=288 tests, a calibration curve was produced. Kit-supplied calibrators for all 4 analytes were run in duplicate on the first plate of each batch, and from this data, a four-parameter logistic curve was fit to the assayed concentrations. Calibration curve validity was then assessed by running two known controls (low and high) included in the kit for each analyte (see section 4.1.3.2).

4.1.3.2 Individual Assay Quality Control

Each assay kit contained reagents for assaying 96 samples plus two known (low and high) concentration controls per analyte. Exact concentrations of controls varied from lot to lot, but typical values are listed in **Figure 5**.

Analyte	Low Control (pg/ml)	High Control (pg/ml)
NfL	10	925
Tau	4	200
GFAP	27	1500

Figure 5. Typical Low and High Control Concentrations

Figure 6 lists the lower and upper limits of detection (LLOD; ULOD) for each analyte when run at a 1:4 dilution, as well as the coefficient of variation (CV = standard deviation ÷ mean), expressed as a percent, for each of the control samples for each assay.

Analyte	LLOD (pg/ml)	ULOD (pg/ml)	Low Control CV (%)	High Control CV (%)
NfL	1.864	1,448	10.72	7.67
Tau	0.616	312	7.36	9.92
GFAP	4	3,260	9.72	9.64

Figure 6. Levels of Detection and Assay Ranges per Analyte

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 concentration mean and coefficient of variation (CV) based on those assays are tabulated in **Figure 7**. When analyte concentrations exceeded acceptable parameters, the Simoa system was investigated and repaired.

Analyte	Serum Mean (pg/ml)	Serum CV (%)	Plasma Mean (pg/ml)	Plasma CV (%)
NfL	9.50	9.25	7.50	10.97
Tau	0.20	50.66	5.20	13.28
GFAP	92.90	11.14	59.70	12.54

Figure 7. Plasma and Serum Quality Control Values

5. External Quality Control

5.1 Reliability

Within a race/ethnicity- and sex-stratified random sample of 132 Add Health participants among whom venous blood was collected twice, on average 13.2 (95% confidence interval: 12.0-14.3) days apart, typically by the same FE and at approximately the same time of day, the reliability of all analytes were estimated as an intra-class correlation coefficient (ICC, 95% confidence interval) (**Figure 8)**. The estimates suggest that within Wave VI, NfL and GFAP are highly reliable. Tau is less so, both within Wave VI and compared to Wave V.

Measure	N	ICC	95% CI
NfL	132	0.96	(0.95,0.97)
Tau	131	0.62	(0.52,0.73)
GFAP	132	0.99	(0.99,1.00)

Figure 8. Reliability of Analytes

5.2 Dependence on Handling Intervals

The NfL, tau, and GFAP concentrations were related to blood handling intervals. For example, median tau concentrations were highest in deciles 1-2 and lowest in deciles 9-10 of the blood collection-LCBR receipt interval (**Figure 9**). In other words, tau varied inversely with pre-analytical handling time, an observation consistent with the possibility of proteolytic degradation. *Warning: Users should therefore recognize the dependence and use tools designed to properly address it (e.g., restriction, stratification, adjustment) in corresponding analyses*. To that end, all handling intervals between blood collection, centrifugation, shipment, receipt, and assay can be found in the Add Health Home Exam Health and Quality Control Metrics data, code book, and user guide.[6]

	Collection - Receive Interval* Decile									
Median	**1 (n=550)**	**2 (n=550)**	**3 (n=549)**	**4 (n=551)**	**5 (n=548)**	**6 (n=547)**	**7 (n=549)**	**8 (n=549)**	**9 (n=551)**	**10 (n=547)**
Interval (hr)*	19.2	21.7	23.0	23.8	24.6	25.6	38.9	44.7	48.4	72.2
NfL (pg/ml)	8.2	7.8	8.0	7.8	7.6	7.8	8.0	8.0	8.0	8.1
Tau (pg/ml)	4.4	4.6	4.1	4.2	4.1	3.9	4.3	4.1	3.8	3.7
GFAP (pg/ml)	76.8	78.8	79.7	80.9	79.0	75.7	78.4	78.0	79.1	78.4
*H6BLOODREC										

Figure 9. Median NfL, Tau & GFAP Concentrations by Collection-Receive Interval Decile

6. Constructed Variables

All analyte concentrations were categorized based on their corresponding limits of detection (LODs) and extrapolation beyond them, then flagged as tabulated below (**Figures 9-12**). *Warning: Users should recognize extrapolated concentrations as such, and exercise caution if choosing to work with them. Moreover, theoretical and computational recommendations for properly analyzing left- and right-censored concentrations falling beyond limits of detection under both frequentist and Bayesian frameworks can be found elsewhere.*[7]

6.1 Flag Indicating the NfL Concentration Type [H6NFLFL]

Code	Description
1	NfL concentration is below the lower LOD (< 1.864 pg/ml) – missing
2	NfL concentration is below the lower LOD (< 1.864 pg/ml), but extrapolated
3	NfL concentration is within the LODs (1.864 - 1448 pg/ml)

Figure 9. Flag Indicating the NfL Concentration Type

6.2 Flag Indicating the Tau Concentration Type [H6TAUFL]

Code	Description
1	Tau concentration is below the lower LOD (< 0.616 pg/ml) – missing
2	Tau concentration is below the lower LOD (< 0.616 pg/ml), but extrapolated
3	Tau concentration is within the LODs (0.616 – 312 pg/ml)
4	Tau concentration is above the upper LOD (> 312 pg/ml), but extrapolated

Figure 10. Flag Indicating the Tau Concentration Type

6.3 Flag Indicating the GFAP Concentration Type [H6GFAPFL]

Code	Description
1	GFAP concentration is below the lower LOD (< 4.00 pg/ml) – missing
2	GFAP concentration is below the lower LOD (< 4.00 pg/ml), but extrapolated
3	GFAP concentration is within the LODs (4.00 – 3260 pg/ml)

Figure 10. Flag Indicating the GFAP Concentration Type

6.4 AntiParkinson and Alzheimer's Disease Medications Use [H6EPKALZ]

Use of a prescription medication in the past four weeks in one or more of the therapeutic classes listed in **Figure 11** 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
057-066-***	Antiparkinson agents	H6EPKALZ
057-066-205	Anticholinergic antiparkinson agents (excluding medications containing diphenhydramine as the sole anticholinergic antiparkinson agent as an active ingredient)	
057-066-206	Miscellaneous antiparkinson agents	
057-066-276	Dopaminergic antiparkinsonism agents	
057-080-***	Miscellaneous central nervous system agents (containing an ergoloid mesylate, glutamate receptor antagonist, or amyloid-targeting monoclonal antibody as an active ingredient)[1]	
057-313-***	Cholinesterase inhibitors	

[1]Active Ingredients:

- Ergoloid mesylates
- Memantine
- Aducanumab
- Lecanemab

Figure 11. AntiParkinson and Alzheimer's Disease Medications Use

Therapeutically classified use of prescription medication in particular classes may confound biomarker-based estimates of Parkinson and Alzheimer's disease. 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.[8,9]

7. The Neurodegeneration Biomarker Data File (bneuro6.sas7bdat)

7.1. Structure

The structure of the disseminated neurodegeneration biomarker 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 neurodegeneration biomarker 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
H6NFL	Neurofilament Light (NfL, pg/ml)
H6TAU	Tau Protein (pg/ml)
H6GFAP	Glial Fibrillary Acidic Protein (GFAP, pg/ml)
H6NFLFL	NfL Concentration Type
H6TAUFL	Tau Concentration Type
H6GFAPFL	GFAP Concentration Type
H6EPKALZ	AntiParkinson/Alzheimer's Disease Medication Use

8. References

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