Biomarker Collection – HS&B:80-2021 and NLS:72-2025

Blood and saliva samples were first collected in the 2021 follow-up of HS&B:80 and in the 2025 follow-up of NLS-72. See Table 1 for blood and saliva response rates and projections.

We have partnered with ExamOne, a professional biomarker collection company, and the Advanced Research and Diagnostic Lab (ARDL) at the University of Minnesota to collect and process data. ARDL receives all blood and saliva samples for processing, assaying, and storage for HS&B:80-2021 (HS&B) and NLS:72-2025 (NLS-72). See Table 3 for a complete list of HS&B and NLS-72 assays.

HS&B:80-2021

During HS&B, venous blood draws and saliva collection occurred in participant's homes with an ExamOne phlebotomist or nurse, and participants who did not consent were mailed a saliva kit for self-collection. See Table 2 for physical measurements collected in HS&B.

NLS:72-2025

For NLS-72, our interviewers complete in-home assessments which include physical measurement and saliva collection, and participants are asked to consent to a venous blood draw by an ExamOne phlebotomist or nurse at a later date. See Table 2 for the additional physical measurements collected in NLS-72.

Table 1. HS&B:80-2021 and NLS:72-2025 Biospecimen Collection Response Rates

	HS&B	NLS-72*
Venous blood	4,360	2,640
Saliva	6,260	6,730

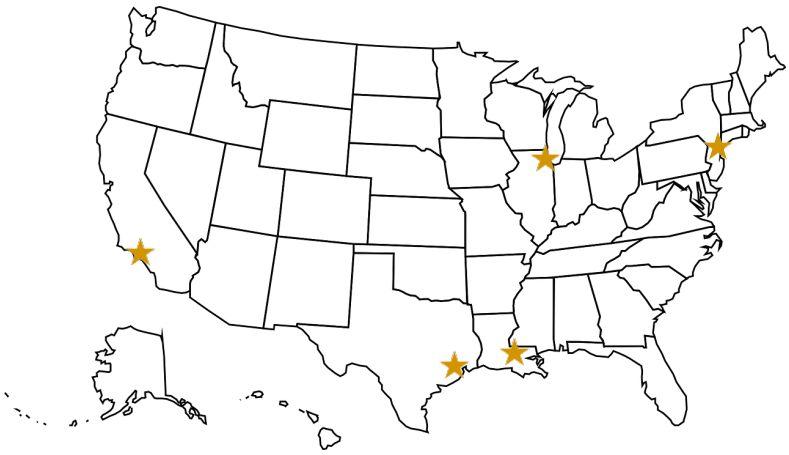
*Projected numbers for NLS-72

Table 2. HS&B:80-2021 and NLS:72-2025 Biomarker Collection

Measure	HS&B	NLS-72
Venous blood	X	X
Saliva	X	X
Blood pressure	X	X
Pulse	X	X
Height	X	X
Weight	X	X
Hip circumference	X	X
Waist circumference	X	X
Grip strength		X
Hearing		X
Timed chair stands		X

NLS:72-2025 and MRI Data Collection

MRI data are collected for the first time during the NLS:72-2025 follow-up. Selected participants who consent to be contacted for future studies are asked to participate in an MRI scan session. The MRI centers, which are geographically distributed to reflect the diversity of the NLS-72 cohort, are as follows:



- Zuckerman Institute at Columbia University (New York, NY)
- Rush University Medical Center (Chicago, IL)
- Pennington Biomedical Research Center at Louisiana State University (Baton Rouge, LA)
- University of Houston (Houston, TX)
- University of California, Los Angeles (Los Angeles, CA)

The MRI session includes a standardized and harmonized series of sequences, following guidance from the ADNI4 protocol. These scans are used to visualize different aspects of brain structure and function. The total scan time is less than one hour.

The MRI imaging protocol is as follows:

1. 3-Plane Localizer
2. Accelerated Sagittal MPRAGE (3D T1)
3. Sagittal 3D FLAIR
4. Sagittal 3D T2 Space Weighted
5. Axial 2D/3D ME T2 GRE
6. Axial 3D PASL / pCASL
7. Axial DTI PA
8. Axial DTI AP
9. fMRI

The raw MRI data are stored in the Laboratory of Neuro Imaging (LONI) at the University of Southern California for warehousing, initial quality control, and future distribution to the wider community. Before neuroimaging data are made available via LONI's Image and Data Archive (IDA) for analysis, defacing software developed by the Mayo Clinic is applied to all scans to remove any personal identifying information and protect NLS-72 participants' confidentiality.

De-identified data will be used to derive measures of neurodegeneration and cerebrovascular health. Primary measures of neurodegeneration will comprise mean cortical thickness in AD/ADRD 'signature' regions and hippocampal and entorhinal cortex volume. For measures of cerebrovascular health, the outcomes will include total WMH volume, presence of infarct, presence of microbleed, and regional cerebral blood flow.

Table 3. HS&B:80-2021 and NLS:72-2025 Blood Assays and Saliva Testing

Physiological:	**HS&B Assays**	**NLS-72 Assays**
Adiponectin		X
Albumin	X	
Alkaline phosphatase	X	
Basophils %, Basophil Count	X	X
B-type natriuretic peptide, N-terminal pro (NT-proBNP)	X	
C-Reactive Protein (high sensitivity)	X	X
Creatinine	X	
Cystatin C	X	
D-dimer	X	
Eosinophils %, Eosinophil Count	X	X
Ferritin		X
Fibrinogen	X	
Glucose, Fasting	X	
HDL, LDL, and Total Cholesterol	X	X
Hematocrit	X	
Hemoglobin (HbA1c), Hemoglobin (pg)	X	X
Insulin and Insulin-like growth factor (IGF-1)	X	
Iron		X
Lymphocytes %, Lymphocyte Count	X	X
Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration	X	X
Mean Corpuscular Volume	X	X
Platelet Count and Mean Platelet Volume (MPV)	X	X
Monocytes %, Monocyte Count	X	X
Neutrophils %, Neutrophil Count	X	X
Platelet-derived growth factor (PDGF-AA)		X
Platelet Distribution Width (PDW)	X	
Placental growth factor (PlGF)	X	
Red Blood Cell and White Blood Cell Count	X	X
Red Cell Distribution Width	X	X
Transforming Growth Factor beta (TGF_b)	X	
Triglycerides	X	X
Urea Nitrogen (BUN)	X	
Vitamin D		X
Genetic Testing:		
APOE Genotyping	X	X
DNA methylation	X	X
Whole Genome Sequencing (subsample)	X	
GWAS		X

Table 3. Continued

Inflammatory and Immune Markers:	HS&B:80 Assays	NLS:72 Assays
CMV IgG4	X	
IL-10	X	
IL1RA	X	
IL-6	X	
TNFRII	X	
Soluble receptor for advanced glycation end-products (sRAGE)	X	
Soluble urokinase plasminogen activator receptor (suPAR)	X	
Olink Target 96 Neurology Panel	X	X
SARS CoV-2 Antibodies	X	
Neurological and Neurodegenerative:		
Aβ42/40 ratio	X	X
Neurofilament light (NfL)	X	X
p-tau 181	X	X
Plasma total tau		X
Glial Fibrillary Acidic Protein (GFAP)	X	X
Growth Factors and Hormones:		
Basic fibroblast growth factor (bFGF)	X	
fT4 (reflex if TSH is out of range, estimate 25% of cohort)		X
Growth Differentiation Factor-15 (GDF-15)	X	
Thyroid Stimulating Hormone (TSH)		X
VEGF	X	
Environmental Exposure:		
Arsenic	X	
Cadmium	X	
Chromium	X	
Cobalt	X	
Copper	X	
Lead	X	
Manganese	X	
Selenium	X	
Thallium	X	
Zinc	X	

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