CLEANED/DERIVED VARIABLE METADATA TOP SHEET

For Submission to the NSHD Scientific Support Team

Date of submitting documentation	11/07/2019
Categories of variables*: **(may be more than one)**	Simoa blood biomarkers tested on phase 1 plasma and serum samples from the LWBL/UKDRI biobank
Summary of work undertaken	Source data transferred to Stata file Missing data – labelled as -99. Methods section below
Source data file(s)	Excel
Date source file(s) created:	11/07/2019
Names of source variables	SerumNFL Tauplasma Ab42plasma Ab40plasma Ab4240ratioplasma plasma_available serum_available
Syntax provided	No
Location of syntax file	N/A
Date syntax file created:	N/A
Format of syntax	N/A
Output variables (please list names of new variables created)	• same as source variables
Output data file provided	Yes
Date output file created:	07/06/2019
Location of output file	N:\Test Data and Video Files\Phase 1\3_Cleaned Data\Insight46_Simoa blood biomarkers_phase_1_final_20190711.dta
Format of output file	Stata data file (.dta)
Documentation provided	N/A
List any papers in which cleaned/derived variables have been used	1. Blood and cerebrospinal fluid biomarkers in Alzheimer's disease – from clinical to preclinical cohorts. PhD thesis (Ashvini Keshavan). 2. Plasma amyloid, tau and serum neurofilament light chain in Insight 46 – associations with cognition and brain imaging. AAIC 2019 poster (Keshavan et al.)

	3. The Insight 46 Cohort – Relationships with Cerebral PET Amyloid positivity Using Two Independent Methods for Plasma Amyloid Beta Measurement. AAIC 2019 Featured Research Session Oral presentation. (Schott et al.)

Methods (taken from reference 1 above):

Serum was chosen for NFL analysis and plasma for analysis of the other three biomarkers (total tau, Aβ40 and Aβ42) as per the recommendations from our prior publication detailing plasma vs serum comparisons of quantifications of Simoa blood biomarkers in other samples (Keshavan et al. 2018, Alzheimer's and Dementia, Diagnosis, Assessment and Disease monitoring).

For serum NFL analysis: a single 500 µL aliquot of serum for each individual was thawed directly to room temperature over 1 hour and vortexed for 2 seconds to ensure thorough mixing. 200 µL was pipetted into a 1.5 ml polypropylene centrifuge tube and centrifuged at 13 000 g for 10 minutes, as per the kit manufacturer's recommendation; the remaining 300 µL was replaced into -80 ºC in the original cryovial. After the 200 µL was centrifuged, 130 µL of the supernatant was pipetted onto the plate for analysis in duplicate. If the coefficient of variation (CV) across the duplicates was >15% or no value was returned for either, the procedure above was repeated at a later date, employing one additional freeze-thaw cycle by starting with the 300 µL volume that was in the original cryovial. At this point all 500 individuals who had blood sampling had a serum NFL value quantified with a CV <15%.

For plasma Aβ40 and Aβ42 analysis: a single 500 µL aliquot of plasma for each individual was thawed directly to room temperature over 1 hour and vortexed for 2 seconds. 300 µL was pipetted into a 1.5 ml polypropylene centrifuge tube and centrifuged at 13 000 g for 10 minutes as per the kit manufacturer's recommendation; the remaining 200 µL was replaced into -80 ºC in the original

cryovial. After the 300 µL was centrifuged, 100 µL of the supernatant was pipetted onto each of two plates for analysis in duplicate, capitalising on the ability to load two different reagent kits at a time on the HD-1 analyser. When plates of samples for analysis of Aβ40 and Aβ42 were prepared in this way, the plate containing samples for Aβ40 was always analysed first, and that containing samples for Aβ42 was analysed second. The CV across the duplicates was <15% for all samples assayed for Aβ40 but for some samples assayed for Aβ42 the CV was >15% or no value was returned. In this case the procedure above was repeated at a later date, using a fresh 500 µL aliquot of plasma and pipetting out and centrifuging 200 µL then pipetting 100 µL of the supernatant onto the plate for Aβ42 analysis.

For plasma t-tau analysis: if only one aliquot of plasma had been used for Aβ42, the second (fresh) 500 µL aliquot of plasma for each individual was used for assaying total tau and the steps undertaken to prepare the sample thereafter were identical to those described above for serum NFL. However, if the Aβ42 assay was being repeated on the second aliquot, then on the same thaw of this sample, after vortexing for 2 seconds, 200 µL was pipetted into a separate 1.5 ml polypropylene centrifuge tube and used for plasma t-tau analysis in parallel. In this situation, the plate of samples for analysis of Aβ42 was analysed first and the plate for analysis of t-tau second. If the CV across duplicates was >15% on the first analysis of t-tau, the analysis was repeated at a later date, employing one additional freeze-thaw cycle by starting with the 300 µL volume that was in the original cryovial.

By this method, all samples analysed for plasma Aβ40 and Aβ42 underwent one freeze-thaw cycle; 59 samples analysed for plasma t-tau and 72 samples analysed for serum NFL underwent two freeze-thaw cycles. This procedure was deemed to be acceptable based on the evidence that up to four freeze-thaw

cycles do not affect measured plasma t-tau or serum NFL concentrations for these assays (Keshavan et al. 2018, Alzheimer's and Dementia, Diagnosis, Assessment and Disease monitoring).

For each blood biomarker, all aliquots were assayed using the same batch of reagents excepting the last plate of samples, which were those requiring repeat analysis. Every plate was analysed according to its own calibrators (made from the stock solution provided in the kit), and included two run validation controls also made from this stock. The measured values of the two controls used in each case had an inter-plate CV of <30% so all plates were deemed acceptable for inclusion in the statistical analysis. Details of the commercially available Simoa total tau 2.0, NF-light, Aβ40 and Aβ42 assays are given in the kit inserts and they were all used as per the manufacturer's instructions. All measurements were conducted by the same individual (Dr Ashvini Keshavan) on the same automated HD-1 analyser (Quanterix) at the UK DRI laboratory at UCL.

Where no aliquot of plasma or serum was available in the biobank for a given individual (due to failure of blood sampling or incomplete blood sampling) the variables plasma_available and serum_available indicate this. For the blood biomarker variables, the code -99 indicates:

1. in the case where an aliquot was available: the coefficient of variation across the duplicates was above 15% and therefore the quantification was unreliable.
2. in the case where an aliquot was not available: no quantification was performed.

It is recommended that prior to making a request for any of these variables, researchers discuss the plan with Dr Ashvini Keshavan.