WHI Long Life Study: Synopsis of Blood Protocol

- The Long Life Study blood processing/shipment protocol assumes that the samples will be collected in a home or community setting at locations all across the US and thus requires a standardized protocol that can be reliably followed in the field. Employees of the project's data collection company (Examination Management Services, Inc.) will collect the blood samples as part of the in-person visit, transport them for initial processing to a location with an appropriately calibrated centrifuge, perform initial (and minimal) sample processing *within 2 hours* of the draw, pack samples for shipment according to a detailed procedure, and ship samples and forms for final processing to a central lab (FHCRC Specimen Processing Laboratory, SPL). The time between sample collection and final processing will not exceed 36 hours.

- On the day SPL receives a sample shipment, they will (1) inventory the package and assess the quality of the packaging, (2) deliver the 2 ml EDTA vial to the Seattle Cancer Care Alliance (SCCA) hematology lab for immediate CBC testing, (3) deliver the PAXgene vial to the Fred Hutchinson Cancer Research Center Public Health Sciences Biomarker Lab for batched RNA/microRNA extraction within three months, and (4) process the plasma and serum vials. The 'products' of the SPL 'day of receipt' processing will be parent and daughter aliquots of serum, EDTA plasma, and unwashed packed red blood cells to be frozen at -80 degrees C for subsequent shipment to the WHI Biorepository (Fisher BioServices, Rockville, MD). Also on the day of receipt, SPL will draw off the buffy coat from the EDTA vial and freeze it for batched DNA extraction within ~one month. Samples for CVD biomarkers will be shipped within approximately one month of draw from Fisher to the University of Minnesota Fairview Laboratory. All transport, processing, storage, and shipment will be temperature controlled according to a detailed procedure.

- Data on pre-analytic variables (e.g., time of draw, quality of draw, needle gauge, time of initial processing, time of central lab receipt, quality of packaging as received by central lab, time aliquots are frozen) will be recorded at the point of draw, initial processing, and final processing.

Table 1: Draw Order, Vial Type, Purpose

Draw Order	Vial Type	Purpose
1	**EDTA separator tube (pearl)** 8.5 ml Plasma	Proteomics, metabolomics; Separator tube increases the protection against changes with delayed separation that might affect certain assays.
2	**EDTA tube (lavender)** 2 ml Whole blood	Immediate hematological assays
3	**EDTA tube (lavender)** 10 ml Plasma, DNA, red cells	Wide range of current and future DNA-based and protein assays, e.g., proteins, products of metabolism, genotyping, sequencing, telomere length
4	**Serum separator tube (red/gray)** 8.5 ml Serum	Wide range of clinical chemistry and future assays; Separator tube increases the protection against changes with delayed separation that might affect certain assays
5	**PAXgene tube** 2.5 ml Stabilized RNA	Extracted total RNA (>18 nucleotides, including microRNA) for gene expression analyses, such as real-time RT-PCR and microarray analysis

Table 2: Blood to Biorepository

Blood Product	Volume
Extracted DNA	~ 90 ug (in 2 aliquots)
Extracted RNA	~ 5 ug (in 2 aliquots)
ETDA Plasma (separator tube)	1 x 1.8 ml; ~8 x 0.25 ml
EDTA Plasma (standard tube)	1 x 1.8 ml; ~8 x 0.25 ml
EDTA Unwashed Lysed Red Cells	1 x 1.8 ml; ~6 x 0.25 ml
Serum (separator tube)	1 x 1.8 ml; ~8 x 0.25 ml

Table 3: Limitations of Blood Protocol

Known Limitations	Details
RNA stability uncertain	The long-term freeze/thaw stability of extracted RNA is uncertain, though preliminary assessments show good stability.
Measurement of labile proteins	A special tube with protease inhibitors would be ideal for proteomics. (EDTA does a pretty good job of preserving labile proteins.)
EDTA in the serum	Due to its importance, EDTA vials are first in the draw order, which increases the chances of EDTA contamination of the serum vial.
No chance for PT, PTT	Requires citrated plasma, and requires immediate testing. Funds not available.
No platelet-poor plasma	Platelets start to release compounds into plasma soon after draw. Immediate centrifugation, not possible with this protocol, and other specialized processing produce the most accurate results.
Possible effect of separator gel	Pre-2005 vials had problems. Current vials, if used according to protocol, markedly improve serum and plasma analyte stability and increase serum and plasma yield.
Glucose depletion	Any exposure of the serum or plasma to RBCs, even at refrigerated temperature, could possibly cause some amount of glucose depletion.
No glucose challenge	Some investigators would prefer a 1-hour post glucose challenge draw in lieu of a fasting draw.

Table 4: Assays performed as part of the WHI Long Life Study

Assay	Details
Complete Blood Count	RBC count WBC count Platelet count Hemoglobin Hematocrit Auto-Differential RBC, WBC, and Platelet parameters
Biomarkers (serum)	Glucose Insulin CRP Creatinine Triglycerides Total cholesterol HDL LDL