04/09/2026 Women's Health Initiative – Lab Working Group policy and procedures

The Lab Working Group (LWG) is a group of scientists affiliated with the Women's Health Initiative (WHI) with interest and/or training in lab science that convenes regularly via email or teleconference to assess the repository inventory and utilization, conduct preliminary reviews of lab tests proposed by investigator-initiated ancillary studies, review the quality of assay results, and make repository-, lab- and assay-related decisions as needed.

<u>Current LWG members:</u>

- Jacques Rossouw, NHLBI (chair)
- Andrea LaCroix, UCSD
- Alex Reiner, CCC
- Paul Lampe, FHCRC
- Li Jiao, HCPH

<u>Reviewing proposals, mods, revisions</u>

Ancillary studies requesting access to biospecimens are initially reviewed by the LWG. Review of biospecimen studies involves assessing feasibility (i.e., availability of requested specimen by outcome category), efficient use of specimen, impact on the biorepository, quality control matters, and compatibility with the current portfolio of WHI core biospecimen studies and approved ancillary studies.

Modifications and revisions to an ancillary study's biospecimen usage are also reviewed by LWG.

Reviews are performed off-call, via email. LWG members vote "approved" or "not approved" and are provided the opportunity to give comments. LWG reviews and comments are anonymized and placed on the study Fact Sheet for the ASC and SC to take into account during their review.

<u>Biorepository management:</u>

Biorepository oversight: The LWG provides general guidance regarding the biorepository (Fisher Bioservices). The group is consulted if there are issues that arise at the biorepository affecting the quality of the samples, such as freezer malfunctions or sample mishandling. They provide guidance during periods of change at the biorepository (e.g. consolidation, re-location, etc.), or new biospecimen collection projects (e.g. Long Life Study).

Reservations: As of 5/8/13, no reservations are placed on biospecimen for approved ancillary studies.

Holds: As of 6/17/16, there are no future holds on WHI biospecimens. There are no BAA holds as of the completion of BA25. As of 02/26/2025 there is no hold on DNA.

Volumes available to ancillary studies: Parsimonious use of specimen is an important consideration in review of AS proposals. Without significant scientific justification, ancillary studies are limited to the

following samples volumes (including any necessary “dead volume”, or padding) from a given specimen collection time point:

- 0.25 ml serum, EDTA plasma, citrate plasma, or red blood cells
- 0.5 ml urine (only available for participants from Bone Mineral Density clinics)
- 1-2 ug DNA (depending on the application)
- 0.5 ug RNA (only available for Long Life Study participants)
- Whole blood: does not exist in our repository
- Buffy coat: not available for distribution

Small volume requests, (<100 µL): When studies request smaller sample volumes (~50 µL), sending them 500ul aliquots causes precious sample wastage. To address this, the LWG approved the addition of a second freeze–thaw cycle for 500 µL aliquots to fulfill these smaller-volume requests. The remaining ~450 µL portion, after the second freeze-thaw, will be returned to Fisher for storage. By following this process, the repository inventory will not expand.

Aliquoting and shipping costs will be charged to the ancillary study, so there will be no additional expense to WHI. For all future requests involving smaller volumes of the same samples, the aliquot with the additional freeze-thaw cycle will be used first, before opening a fresh one freeze-thaw vial. This approach helps preserve the integrity of the remaining samples.

If a study requires fewer freeze-thaw cycles, it will need to provide justification to the ASC. DataOps will proactively inform relevant studies about the second freeze-thaw status as early as possible to manage expectations.

Additionally, in ancillary studies containing samples with additional freeze thaw cycles, WHI will attempt to include blinded samples with similar number of freeze thaw cycles.

Specimen processing: To maintain consistency in handling, all specimen processing (DNA extraction and specimen aliquoting) occurs in the central WHI lab (Biospec Process Biorepository [BPB]). WHI does NOT accept leftover sample from testing labs back into the repository.

Changes to sample handling procedures must be evaluated and approved by LWG.

***DNA extraction*:** The current LWG approved DNA extraction procedure (since 2008) is the manual 5-Prime procedure. In the past, WHI has used 3 other methods of extraction: Bioserve, salt precipitation, and phenol/chloroform. When DNA is extracted, an aliquot is made for the testing lab and the remaining volume is returned to the repository in 2 parent vials. If the total amount of DNA extracted is < 40 ng, it will all be placed into one parent vial. If the total volume is greater than can fit into two parent vials (3.6 ml), the remaining extracted DNA is discarded. DNA subs are no longer routinely made for long term storage to control the size of the repository. DNA subs have also been be problematic because they tend to evaporate in long term storage.

- ***DNA quantitation*:** The current LWG approved method of quantifying DNA is by fluorescence (Picogreen). 260/280 OD ratio is not obtained. Prior to 2007, WHI DNA concentration was measured spectrophotometrically with the 260/280 OD ratio. WHI switched to Picogreen because it was determined that the OD ratio inflated the concentration readings. Previously OD’d samples were not re-quantitated.
- ***DNA aliquoting*:** To ensure that sufficient sample arrives in the testing lab, WHI does not generally distribute aliquots that are < 1 ug and does not generally dilute DNA to a concentration less than 50 ng/ul. Some DNA samples are less than 50 ng/ul upon extraction.

They will be provided as-is if they meet the testing lab's minimum requirements (WHI will not re-concentrate DNA). If a sample was quantitated using the OD ratio, twice as much will be provided to ensure there is enough DNA.

- ***RNA aliquoting***: Please refer to the RNA specimen usage plan, approved by the LWG on 3/5/15: R:\Biological Measurements\Blood\Labs - Core\Song - FHCRC\RNA\RNA Specimen Usage Plan.docx
- ***Blood and Urine aliquoting***: A parent vial is only thawed once. An aliquot is made for the testing lab and the remaining volume is distributed into 500 ul sub-aliquots for storage at -80C for future use. Subs are not further aliquoted to avoid freeze/thaw and control the size of the biorepository. Exceptions have occurred in the past, and the additional freeze-thaws are noted in the database. Special requests from the testing lab for WHI to further aliquot the subs can generally by accommodated, but excess volume from subs (if any) is not generally returned to the biorepository.

  Note (6/23/21): In cases where a lab is requesting a small volume (ex. 50ul) of a valuable sample, LWG has approved to add another freeze thaw to the sample (ex. aliquoting 50ul for the lab and saving 450ul for our inventory)? This plan will only apply to the cases where the lab has no strict requirement of minimum freeze thaw.

Pulling/processing priorities: Unless baseline DNA is specifically requested, buffy coats from time points other than baseline are prioritized for extraction due to a higher rate of baseline DNA extraction failure (serum labeled as buffy, poor collection procedures, etc.).

In the interest of managing the size of the repository, blood and urine subs are generally prioritized for pulling over new aliquots from parents even if this means some sample waste (e.g. 100 ul is needed at the testing lab, but a full 250 ul aliquot is sent).

Quality Control:

Blind duplicates: WHI includes blind duplicates as quality control samples in all sample pulls, as follows:

- DNA: Studies requesting DNA samples are required to include quality control samples: 5% of the total number of participant samples (2.5% blind duplicate pairs).
- Urine and RBC: WHI includes 10% blind duplicate QA samples (5% pairs) with all RBC and urine samples.
- Plasma and Serum: WHI includes 10% blind duplicate QA samples (5% pairs) in studies requesting ≤1,000 participant samples and 5% blind duplicate QA samples (2.5% pairs) in studies requesting >1,000 participant samples.

Note (5/16/22): Requests for modification of blind duplicate numbers will be considered based on QC data from the testing lab for assays being performed (data from vendors will not be considered.)

For the blind QC samples, WHI uses blood samples from women who consented to the screening blood draw but were not enrolled or randomized into any WHI component. The samples are relabeled to provide blinded duplicate samples that are included in all batches of assays. These individuals are different than the individuals selected for a particular study.

QC Reports: QC reporting is described briefly below. Please refer to the Ancillary Study QC Reports document for full details: R:\Dataops\SOPs\QC Reports_012819.docx.

- ***Standard Blood/ Urine Assays:*** The correlation coefficient and the average coefficient of variation % is computed based on the test results for these samples and reported to the PI.
- ***Metabolomics:*** Metabolomics studies are asked to submit a QC report following guidelines the LWG developed in consultation with metabolomics expert, Daniel Raftery, PhD (University of Washington).
- ***DNA Assays:***
  - For smaller genotyping studies (e.g. candidate genes, limited SNPs), the WHI-CCC will produce a SNP concordance report that assesses the study's (1) correct identification of the blind duplicates, (2) failure to identify blind duplicates, and (3) unexpected duplicates among all samples provided.
  - For larger genotyping studies (e.g. GWAS, sequencing), the PI is to report to the WHI-CCC all duplicates identified. From this list, the WHI-CCC will confirm the correct/incorrect identification of the blind duplicates and any unexpected duplicates.

Ancillary Study QC review: The LWG reviews QC reports for ancillary studies if the correlation coefficient for one or more assays is less than 0.9. For Metabolomics studies, the threshold for LWG review is a CV greater than (a) 20% for a metabolite in a Global Metabolomics study, (b) 5% for Targeted Metabolomics, or (c) 10% for Lipidomics.

Based on the blind QC results and additional information from the PI and testing lab, the LWG decides whether or not to approve the assay results for release to the investigator's dataset. Ancillary study QC review is done during the quarterly LWG call and/or via call email follow up.

QC pools: Pooled samples of serum, citrated and EDTA plasma were created close to the start of WHI enrollment to be tested for core analytes and CVD biomarkers alongside participant samples. Core analytes are no longer monitored, but the pooled samples are sent for CVD biomarker testing semi-annually (glucose, insulin, lipids, CRP, and creatinine). Results from these samples allow the LWG to monitor the stability of primary analytes over time.

Sample Processing Quality Control: The adequacy of serum and plasma aliquoting by the Specimen Processing Lab is evaluated by testing a subaliquot from each blind quality control sample for total cholesterol. Similarly, urine samples are tested for sodium. The correlation and average CV% between the members of each blind pair is computed and reviewed by the LWG.

RNA Specimen Usage Plan

Background:

The WHI LLS RNA samples were collected from LLS participants as part of the LLS Blood Protocol using the PreAnalytiX PAXgene blood tubes, a collection system designed to preserve RNA from whole blood. After collection in participant homes throughout the US, PAXgene tubes were the last of five tubes drawn from each participant, mixed carefully (inverted 8-10 times), kept at room temperature for a minimum of 2 hours post draw, and shipped overnight with cool packs to the Biospec Process Biorepository (BPB). Upon receipt at the BPB, techs froze the PAXgene tubes at -80 degrees C until they could be transferred to the Fred Hutch Public Health Sciences Biomarker Lab, under the direction of Dr. Xiaoling Song, where the vials were kept frozen at -80 degrees C until a sufficient number for an extraction run was assembled.

Within about a month of collection, Dr. Song's lab extracted total RNA, including miRNA, from 7,293 PAXgene tubes using the PreAnalytiX method (*PAXgene Blood miRNA Kit Handbook, Qiagen, 05/2009)* designed for use with the PAXgene blood collection tubes. A qualitative assessment by agarose gel electrophoresis of RNA integrity was done at the time of extraction, with less than 0.6% of samples showing any degradation. The RNA was quantified by NanoDrop and the 260/280 ratio recorded. The 260/280 ratio also assessed the purity of RNA, with a ratio of ~2.0 generally accepted as "pure."

The elution volume of 76 µL of extracted RNA was divided between two RNA 'Parent' vials without further dilution, frozen at -80 degrees C, and shipped overnight on dry ice to the WHI biorepository for long term storage at -80 degrees C until selected for use in a WHI Ancillary Study (AS). The WHI Study Tracking database (WHIST) tracks information about each RNA vial, such as the yield (in µg), the concentration (in ng/µL), any additional comments about the extraction process, and Freeze/Thaw cycle data. Each of 7,126 PAXgene tubes yielded two RNA Parent vials with a concentration of ≥ 5.0 ng/µL and good RNA integrity. (Note: As of February 2015, WHI judges the minimum acceptable concentration of RNA to be 5.0 ng/µL. Were that minimum to be set at 50 ng/µL, a total of 5,767 participants would have adequate RNA concentration.)

The 2010 decision to extract the RNA instead of storing long term the whole blood PAXgene tube at -80 degrees C was based on the following rationale:

- The PAXgene tubes and the PAXgene Blood miRNA Kit extraction method constitute a 'system'. A future upgrade of the extraction method might not be usable with the 2010 PAXgene tubes. WHI did not want to take this risk.
- Dr. Song's experience, and that of others, was that RNA is quite stable in its purified form stored in buffer and at -80 degrees C.
- Qiagen data on long term storage of RNA extracted from PAXgene tubes at the time indicated no significant RNA degradation or changes in transcript levels after storage at -80 degrees C for 50 months.
- The NHLBI Project Office wanted the RNA to be immediately usable by ancillary studies.

The 2011 decision to split the extracted RNA into two RNA 'Parent' vials, rather than multiple 'Daughter' vials, was based on Dr. Song's experience. Per Dr. Song (7/19/11): "The Bio-Analyzer integrity tests of RNA samples thawed once (or twice) showed that the samples are fine in our experience. So I think your proposal of making smaller sub- aliquots after the 1st thawing (instead of making more aliquots initially) is a sensible one. Please note though the initial 2 parent aliquots we will be making is only ~38ul each. So, depending on how much will be taken out for the 1st downstream experiment, it may or may not worthwhile to subdivide the leftover."

<u>Quality Assurance (QA) RNA Samples</u>

In spring of 2013, six PAXgene tubes were drawn on each of 5 post-menopausal female volunteers to serve as QA samples. Each QA volunteer's PAXgene tubes was labeled with a different Draw ID number. Three of the Draw IDs from a QA volunteer are considered the 'Real' Draw IDs, and the other three are considered the 'Relabeled' Draw IDs. Each 'Real' Draw ID is paired with a single 'Relabeled' Draw ID to form a Blind Duplicate QA Pair. So, WHI has a total of 30 PAXgene QA Draws and 15 QA Pairs of Draws (i.e., 5 volunteers * 6 Draws ÷ 2 Draws/QA Pair). The QA PAXgene Draws were processed according to the LLS Blood Processing Protocol. Each of the PAXgene tubes drawn from the QA volunteers yielded sufficient RNA volume to be split into two RNA 'Parent' vials. 'Daughter' aliquots from the RNA QA 'Parent' vials will be included among participant RNA samples in ancillary studies to assess the reliability of RNA test results.

Facts about the WHI RNA samples:

1. Total RNA Yield for participants (Ppt) and Blinds with concentrations of >= 5 ng/ul:

Participant Type	AVG YIELD (in µg)	MIN YIELD (in µg)	MAX YIELD (in µg)	AVG CONC. (in ng/µL)	MIN CONC (in ng/µL)	MAX CONC. (in µL)	N
Participant	6.62	0.51	42.59	87.12	6.65	560.4	7126
Blind	7.83	2.54	17.85	103.09	33.48	234.9	30

2. Average Ppt RNA yield <u>per Parent Vial</u> = 3.3 µg
3. Average QA RNA yield <u>per Parent Vial</u> = 3.9 µg
4. Volume <u>per Parent Vial</u> = 38.0 µL

<u>Use of WHI RNA Samples in WHI Ancillary Studies (AS)</u>

1. **<u>First use of a RNA 'Parent' vial</u>**: Upon first selection of a participant's RNA 'Parent' vial for use in an AS, the Parent vial will be included in a Pull for processing according to BPB RNA Processing Protocol (attached, dated 2/18/2015). A 'test aliquot' of the approved amount will be made by BPB for the testing lab, with the concentration normalized per the AS requirements.
   a. *If there is > 20 µL residual volume after preparing the test aliquot, the Parent vial will be split into two Daughter vials for return to the repository – without further dilution.*
   b. *If there is between 5 µL and 20 µL after preparing the test aliquot, the entire residual volume will be placed into a single Daughter vial for return to the repository – without further dilution.*
   c. *If there is < 5 µL after preparing the test aliquot, the residual volume will be discarded.*

2. **<u>Use of a RNA 'Daughter' vial</u>**: Upon selection of a participant's RNA 'Daughter' vial for use in a WHI AS, the Daughter vial will be included in a Pull 'set' of 'existing' vials to be inserted among the newly created test aliquots <u>without</u> incurring another thaw. In this way, the number of thaws will be the same (one) for every sample in a Pull.
   a. The shipping manifest will note the Date of 1st Thaw for each vial in the shipment so that the time since 1st thaw could be used, if needed, in the AS's analysis.
   b. Because the Daughter was re-frozen at the highest possible concentration (to maintain RNA integrity), the actual concentration of each sample in the shipment will be noted on the manifest.

3. <u>Use of RNA 'Daughter' vials before opening the 2nd Parent vial</u>: It will be WHI policy to use Daughter vials, when they exist, before using the 2nd Parent vial.

4. **<u>Number of ASs for which a participant's RNA could be used:</u>** The number of times a participant's RNA could be used in an AS will vary *between 2 and 6* depending on the original yield of extracted RNA and the amount requested by each AS.

5. **<u>Blind Duplicate QA Pairs</u>**: Blind duplicate QA samples will be included among the participant samples in a manner similar to that of the WHI DNA, and the QA samples will be processed exactly as for the participant samples. However, the quantity of QA RNA Pairs is very limited. So, the percentage of QA RNA Pairs will be as follows:
   a. *<u>Studies with ≤400 participant samples:</u> 2% QA pairs (4 QA samples per 100 ppt samples)*
   b. *<u>Studies with >400 participant samples</u>: 1% QA pairs (2 QA samples per 100 ppt samples) up to a*

maximum of 10 pairs (20 samples) per study

These percentages of blind duplicate QA pairs are based on the fact that there will be a maximum of 90 QA pairs that could ever be used in RNA ASs. The table below outlines how the 6 PAXgene tubes drawn from a single QA Volunteer generated *up to* 18 QA pairs of Daughter vials; multiplying by 5 yields a total (*maximum*) of 90 QA pairs of Daughter vials. The bottom line is that WHI can include QA samples in a maximum of 9 studies of 1000+ RNA samples.

PAXgene Vials drawn from one QA Volunteer (example)	**Draw 123**	**Draw abc**	**Draw 456**	**Draw def**	**Draw 789**	**Draw ghi**	**Each QA Volunteer generates:**	**All 5 QA Volunteers generate:**
QA Pairs (in dbase)	123	abc	456	def	789	ghi	3 QA Pairs of PAXgene tubes	15 QA pairs of PAXgene tubes
RNA 'Parents' from PAXgene	123-1 123-2	abc-1 abc-2	456-1 456-2	def-1 def-2	789-1 789-2	ghi-1 ghi-2	6 QA paired 'Parents'	30 QA pairs 'Parents'
RNA 'Daughters' from 'Parent'	123-11 123-12 123-13 123-21 123-22 123-23	abc-11 abc-12 abc-13 abc-21 abc-22 abc-23	456-11 456-12 456-13 456-21 456-22 456-23	def-11 def-12 def-13 def-21 def-22 def-23	789-11 789-12 789-13 789-21 789-22 789-23	ghi-11 ghi-12 ghi-13 ghi-21 ghi-22 ghi-23	18 QA paired 'Daughters' (maximum, depending on the volume of the Parent vials)	***90 QA pairs of 'Daughters' (maximum, depending on the volume of the Parent vials)***

Draft - Ancillary Study QC Reports

<u>Standard Blood Assays</u>

Upon data submission, the following QC reports are generated from Ancillary studies performing standard blood assays.

a) *Blind duplicate Report*
   CCC provides a blind duplicate report to the PI which includes:
   - Participant results average and standard deviation.
   - Blind duplicate correlation, average and max CV's and minimum pull correlation. We are looking into including ICC as well.
b) *PI QC response*
   If any assays had a correlation of less than 0.9, CCC requests a response from the AS PI and the report is reviewed by the LWG.
c) *Testing Lab Internal QC report*
   Includes a brief description of internal QC measures, inter-assay CVs, and anything else notable.
d) *PI QC Report*
   If data was not loaded, CCC reveals paired samples and PI prepares QC report.

i. How data was generated.
ii. Percent missing out of the intended query.
iii. What missingness cut-off was used for selecting analytes.
iv. How many analytes were targeted?
v. Average CV, quality of identification of analytes, and include any additional information you think is important. CV for the run is an important number to report.
vi. For instrument/ run performance, CVs are useful (as well as not transforming the data).
vii. Are there cutoffs (CVs, MS intensities) at which point the run is deemed to not be working well, and the samples need to be rerun?
viii. Provide Intra-class correlation as a scale free measure
ix. List the analytes that are performing poorly, either in the QCs or in the blinded duplicates
x. Types of QCs (pooled sample QC sample and/or reference QC sample) that were incorporated, and how often were they run.

DNA Assays

Upon data submission, the following QC reports are generated from Ancillary studies performing DNA assays;

a) SNP studies
CCC provides a SNP concordance report to the PI.
b) For assays other than SNP's
CCC reveals paired samples, PI compares the results for each pair and reports findings.
c) For GWAS that requires a small set of data for cleaning prior to submitting cleaned data to CCC
The testing lab identifies the samples that matched in the GWAS (prior to receiving any data).

Metabolomics

Upon data submission, Ancillary studies performing metabolomics submit a QC report for LWG review that includes the following:

a) Identify whether global or targeted metabolomics.
b) How data was generated.
c) Percent missing out of the intended query.
d) What missingness cut-off was used for selecting analytes.
e) How many metabolites were targeted?
f) Average CV, quality of identification of metabolites, and include any additional information you think is important. CV for the run is an important number to report.
g) For instrument/ run performance, CVs are useful (as well as not transforming the data).
h) Are there cutoffs (CVs, MS intensities) at which point the run is deemed to not be working well, and the samples need to be rerun?
i) Provide Intra-class correlation as a scale free measure
j) List the metabolites that are performing poorly, either in the QCs or in the blinded duplicates
k) Types of QCs (pooled sample QC sample and/or reference QC sample) that were incorporated, and how often were they run.

Notes for Metabolomics review (per Guidance from Metabolomics Expert- Dan Raftery, 8/31/17 LWG call): Acceptable CV for a metabolite: instrument performance- nothing above 20% for quality control

samples (except if run is very long, running for weeks, since instruments get dirty. Look at shorter run). No need to log transform CVs. A typical CV for global profiling is 20%. Targeted tends to be better (around 5%). Lipidomics are higher (about 10%)