MOP 10

REPEATABILITY AND REPLICATE SUBSTUDY

Version 1

September 13, 2012

1.0 Repeatability Substudy

1.1 Participant Selection

The entire clinic visit will be repeated on 100 volunteers to determine reliability and short-term variability of measurement procedures. For this substudy, all interviews will be re-administered, all procedures (including CT) will be repeated and new samples of blood, urine, and saliva will be collected. The repeatability visit will occur at least two but no more than six weeks from the first visit. Clinical center staff will process these biospecimen samples according to the already established protocol.

Each site will recruit 16-17 volunteers to participate in the Repeatability Study. The Repeatability Substudy participants do not need to be distributed over time (i.e., they may, and should, be recruited as quickly as possible). Selection of participants will reflect the four strata with sites recruiting approximately 2 healthy controls, 3 smokers, 6 mild/moderate cases of COPD, and 6 severe cases of COPD. See table 1 below for exact distribution of participants across sites. Representation of other subgroupings (e.g., gender, age) is advised, but not required, and will be monitored by the GIC**.**

Table 1. Distribution of Participants in Repeatability Substudy Across Sites

	Total	Stratum 1	Stratum 2	Stratum 3	Stratum 4
Columbia	9	1	2	3	3
JHU	8	1	1	3	3
WFU	17	2	3	6	6
UCLA	17	2	3	6	6
UCSF	17	2	3	6	6
MI	16	2	3	5	6
Utah	16	2	3	6	5
Total	100	12	18	35	35

Study Coordinators will offer the Repeatability Substudy to all participants until their site strata quotas are filled.

If a subject would like to participate in both the Repeatability Substudy and the Bronchoscopy Substudy, the visits should be scheduled using the following guidelines:

1) If a subject does both substudies, they should do the Repeatability visit before the first Bronchoscopy Substudy visit (BR1). The window for scheduling the Repeatability Substudy is window is 2-6 weeks after V1.

2) Then they can do the first Bronchoscopy Substudy visit for sputum, for which the window is 2-8 weeks after the last clinic visit.

3) There should be at least 2 weeks between all sputum inductions

1.2 Data Collection Procedures

Clinical centers will use "phantom subject ID numbers" for data collected as part of the Repeatability Substudy. The phantom subject ID* numbers are indistinguishable from other ID numbers, and forms belonging to Repeatability Study participants are entered just as regular study data. The Repeatability Visit Linking Form (RID) is used to match the repeat visit data to the original visit data. This process is described in more detail as follows:

1) Data collected on the repeat visit is entered into the DMS using the phantom subject ID.
2) The repeat clinic visit should be done no sooner than 2 weeks and no more than 6 weeks after the original clinic visit. Coordinators should confirm that participants are still eligible for a study visit at the time of the repeatability substudy (e.g., they are not currently taking antibiotics)
3) The same or different coordinator(s)/technician(s) may be used to collect the data, but he/she should refrain from accessing data from the participant's original visit (i.e., the coordinator/technician should be blinded to the original measurement values).

*NOTE: The phantom subject ID numbers for the Repeatability Substudy differ in concept from the phantom lab ID numbers for the Replicate Substudy (below). In the Repeatability Substudy, the phantom subject ID numbers represent a repeat of the ENTIRE visit on ONE individual repeated. In the Replicate Substudy (below), the phantom lab ID numbers represent a combination of samples collected from multiple individuals so that an entire collection "look like" one participant to the processing lab and for all downstream applications. These distinctions are important to prevent confusion. Study Coordinators should be sure to understand these distinctions and ask the GIC for clarification if there is any confusion.

Data from the original and repeat visit will be analyzed to estimate the reliability of all data collection procedures. Methods for computing reliability coefficients, within-person standard deviations, coefficients of variation, and systematic differences are similar to those outlined in MOP 7 – Quality Control and Quality Assurance.

2.0 Replicate Substudy

To estimate the reliability of laboratory measures, a replicate substudy will be conducted. For

the Replicate Substudy, some participants will provide an additional sample of blood or urine repeated **on the same visit** (this distinguishes the Replicate Substudy from the Repeatability Substudy). In order to ensure that the GIC Biospecimens Processing Laboratory is blinded to the QC process, the additional samples collected as part of the Replicate Substudy are labeled with a *phantom lab ID*. In this case, for the Replicate Substudy, the phantom lab ID represents a "phantom participant." Biospecimens are collected from multiple participants to generate all the samples for the "phantom participant." Forms belonging to the phantom sample are entered into the DMS just as regular study data.

Over the entire study, replicate samples will be obtained on 5% of each specimen type (see table below). Nine participants will be needed to provide a complete set of 9 QC replicate specimens (8 tubes of blood and 1 urine specimen), unless urine is collected from a participant providing a blood specimen replicate. At baseline and year 1 just under half of the participants (45%) will contribute to the pool of replicate samples. In year 3 approximately 40% of participants will contribute to the replicate samples.

Table X. Number of replicates per specimen for Baseline, Year 1, and Year 3 Across All Sites

	Red Top 1	Red Top 2	EDTA 1	EDTA 2	EDTA - 2mL	ACD	PaxGene	P100	urine cups
Baseline	160	160	160	160	160	160	160	160	160
Year 1	151	151	151	151	151	151	151	151	151
Year 3	134	134	134	134	134	134	0	134	134

2.1 Participant Selection

The current informed consent allows for up to 7 tablespoons of blood to be collected. This amount is enough to draw all the necessary blood tubes for the standard protocol and it is enough to also cover the drawing of the replicate samples. The amount (7 tablespoons) is enough to allow up to two replicate blood samples (up to 10 ml each) to be drawn from a single participant, if needed to reach the required 5% for that sample at a given site. Initially coordinators will draw only one additional blood sample. **Therefore, all study participants are eligible for the Replicate Substudy.** Determining which participants to draw replicate samples from will depend on whether the study visit is a baseline or follow-up visit.

2.1.1. Baseline Visits

Because the Replicate Substudy is starting after enrollment has already begun, for baseline replicates only, sites will collect one additional tube of blood and/or a urine sample from **EVERY** participant. The GIC will monitor the collection of the replicates, and when a site has "caught-up" sufficiently to ensure that the Replicate Substudy will be successfully, the sites will be instructed to space out the remaining replicates. The cut-point for when each site is "caught-up" will vary, as sites are recruiting at different rates. If needed sites may be asked to draw up to two replicates from a participant to ensure the required 5% sample is met.

Baseline visits present a particular challenge in that the participant's stratum is not known at the time replicate samples are collected. As with samples collected for the main study visits, if it is determined the participant is ineligible for one of the study strata the replicate samples should be discarded with the rest of the samples.

Collecting samples in this way will result in occasionally discarding some of the lab labels for the blood collection tubes. The GIC will provide the sites with additional blank labels that can be used as replacements. If the technician reaches the aliquoting stage and the participant is ineligible for the study, the site should discard the aliquots and contact the GIC for replacement labels.

2.1.2. Follow-up Clinic Visits (Year 1 and Year 3)

For these replicates sites should collect one additional tube of blood and/or urine from every other participant to fill up a phantom lab ID for the follow-up clinical visits. The GIC will monitor the rate of replicate collection and provide feedback on whether a site needs to increase or decrease the collection frequency.

2.2 Data Collection Procedures

Sites will be provided sets of phantom lab IDs. Each set will represent a complete complement of blood and urine specimens (i.e., each set will represent all the blood and urine specimens that would normally be collected from one participant as part of the main SPIROMICS study; in this case, for this substudy, the samples are collected from MULTIPLE patients over time).

2.2.1 Blood Specimen Collection

Coordinators should proceed with collecting 1-2 additional tubes for every participant, starting with collecting extra red tops. The replicate tube should be drawn after its matched tube before moving to the next sample.

For example, a coordinator needs to draw two additional red top tubes from a participant. The coordinator will draw the first main study red top (i.e., Tube 1) followed by the first replicate red top. The coordinator should then proceed to draw the second main study Tube 2 (also a red top), followed by the second replicate red top. The coordinator should then proceed with collecting the rest of the main study samples.

NOTE: If during the blood draw the coordinator determines that it will be difficult to obtain enough blood to fulfill all of the study requirements, the coordinator should make the decision to not take the replicate for that patient (unless the decision needs to be made after the replicate is already taken, in which case, it should be used as the replicate for which it was drawn).

Coordinators should collect all the blood tubes for a given phantom lab ID before proceeding to the next set of labels.

2.2.2 Urine Specimen Collection

To collect a replicate urine sample, the participant must provide at least a 40 mL specimen at the time of collection. A replicate urine sample may be collected from a participant who also provides replicate blood samples; however, coordinators must finish a given phantom lab ID before collecting another urine sample. For example, upon starting a new phantom lab ID (starting with replicates for blood tubes 1 and 2) the coordinator may collect the replicate urine sample from the participant who provides tubes 1 and 2. The coordinator should not then proceed to collecting urine for a different phantom lab ID until the current phantom lab ID has a complete set of specimens.

2.2.3 Blood and Urine Replicate Processing

When a new phantom lab ID set is started, the study coordinator and/or specimen processing technician should start a NEW set of freezer boxes for the phantom lab ID. Blood and urine specimen replicates must be processed the same way and *at the same time* as the main study specimens. When processing the aliquots, take care to be certain that the replicate tube (belonging to the phantom lab ID labels) is processed into the replicate aliquot. **The replicate samples are placed in the freezer box for the phantom lab ID, <u>not in the freezer box for the main study samples.</u> Starting a new box is critical to blinding the samples.**

Depending on the number of samples collected from each participant it will take between four to nine participants to collect all the replicates for one phantom lab ID. The freezer box of replicate samples should not be shipped to the BSP until all the samples for that phantom lab ID have been collected (i.e., it appears to be a complete box of samples).

The cell lysate and PAXGene tube for a given phantom lab ID should not be sent to the BSP until all the samples for a given phantom lab ID have been collected. Keep the cell lysate and PAXGene tube for a given phantom lab ID separate from the main study lysates and PAXGenes. When all the replicates for that phantom lab ID have been collected and shipped to the BSP the cell lysate and PAXGene for that phantom lab ID may then be shipped with the next regular shipment of those sample types (i.e., mixed in with the main study samples).