

**Indiana University Genetics Biobank
Michael J. Fox Foundation**

TITLE: REFERENCE POOL CREATION

CHAPTER: 3-Processing SOPs

SOP #: IUGB-3-52.01

SUPERSEDES SOP#: N/A

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DIRECTOR APPROVAL: Steve Dlouhy DATE: 6-1-16
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1. REVISION

1.1. N/A – New SOP.

2. PURPOSE

2.1. This Standard Operating Procedure (SOP) provides directions to create reference pools. Indiana University Genetics Biobank (IUGB), BioRep and Tel Aviv Sourasky Medical Center will pool and aliquot samples to create reference pool. Investigators use reference pools in experiments to standardize assay results.

3. SCOPE

3.1. The scope of this procedure includes individuals in the biorepositories for MJFF and other collaborators who will receive reference pools as part of their distributions.

4. MATERIALS

4.1. Consumables

- 4.1.1. Cryovials, 0.5mL (VWR #60828-816)
- 4.1.2. Dry Ice
- 4.1.3. Flask, Glass 200mL or larger (or equivalent glass bottle)
- 4.1.4. Thermal Labels
- 4.1.5. Personal Protective Equipment
 - 4.1.5.1. Lab Coat
 - 4.1.5.2. Disposable Gloves
 - 4.1.5.3. Safety Glasses or Side Shields for prescription glasses
- 4.1.6. Pipette Tips
- 4.1.7. Wet Ice

4.2. Reagents

4.2.1. N/A

4.3. Equipment

- 4.3.1. Freezer, -80°C
- 4.3.2. Label printer
- 4.3.3. Laminar Flow Hood
- 4.3.4. Pipette
- 4.3.5. Racks
- 4.3.6. Rotator, in 4°C Refrigerator

5. PROCEDURE

5.1. OBTAIN SAMPLES

- 5.1.1. Retrieve samples from storage location and place on dry ice immediately.
- 5.1.2. Verify all barcode labels match the query results.
 - 5.1.2.1. A second technician checks for validation that all barcodes match the query results.
 - 5.1.2.2. Both technicians initial and date the exported query results after validation.
 - 5.1.2.2.1. Store document in secure location.
- 5.1.3. Determine number of labels to print based upon the total cryovials to be created.
 - 5.1.3.1. See Appendices for aliquot volumes based upon project and specimen type.
- 5.1.4. Print labels for the aliquots.
- 5.1.5. Label the cryovials with the pre-printed thermal labels.
- 5.1.6. Place the pre-labeled cryovials in a tube rack in a -80°C freezer to chill.

5.2. THAW SAMPLE ALIQUOT

- 5.2.1. Thaw aliquots in a 4°C refrigerator.
 - 5.2.1.1. Aliquots can thaw as fast as 30 minutes.
 - 5.2.1.2. Do not allow the samples to thaw for more than 5 hours in the refrigerator.
- 5.2.2. Record time placed in 4°C refrigerator.
- 5.2.3. Aliquot sample as soon as thawing is complete.
- 5.2.4. Remove from refrigerator and place on wet ice in the Laminar Flow Hood.

5.3. TRANSFER SAMPLE TO FLASK (or bottle)

- 5.3.1. Transfer the pre-chilled tube rack with cryovials from the -80°C freezer to dry ice and place in the Laminar Flow Hood.
- 5.3.2. Mix the thawed sample vial by gently inverting 6-8 times a full 180°.
 - 5.3.2.1. Do NOT vortex or mix by pipetting.
- 5.3.3. Remove and discard the cap from the cryovial into the appropriate biohazard bin per institution guidelines.
- 5.3.4. Place flask (or bottle) in wet ice.
- 5.3.5. Transfer the entire content of the samples to a flask (or bottle) of appropriate size using a pipette.
- 5.3.6. Gently mix the pooled sample by rotating the container for 30 minutes at 4°C.
- 5.3.7. Return the pooled sample to wet ice.
- 5.3.8. Begin aliquoting pooled sample into the cryovials on dry ice based upon the aliquot volumes per project (Appendix A).
 - 5.3.8.1. Mix the pooled samples by inverting the container twice after every 5 aliquots.
- 5.3.9. Make as many aliquots as possible including an aliquot to hold the remaining volume of sample pool if not sufficient to meet designated aliquot size.
- 5.3.10. Verify all barcode labels are correct on the sub-aliquot cryovials.
- 5.3.11. Record time frozen over dry ice.
- 5.3.12. Dispose of the cryovials and flask (or bottle) into the appropriate biohazard bin per institution guidelines.

5.4. STORE SAMPLES

- 5.4.1. Enter aliquot samples into database.
 - 5.4.1.1. Record correct volumes for each cryovial.
 - 5.4.1.2. Record the number of freeze thaw cycle for each specimen.
 - 5.4.1.2.1. Extract data from database and verify the freeze thaw cycle number.
- 5.4.2. Set aside on dry ice the first, last and middle cryovial for QC testing.

- 5.4.2.1. Use 100µL aliquot for Hemoglobin Analysis test, QuantiChrom (IUGB-3-49).
- 5.4.3. Store aliquots in -80°C.
 - 5.4.3.1. Store half of aliquots in back up unit.
 - 5.4.3.2. Store remainder of samples in main freezer.
 - 5.4.3.3. Enter storage location into database.

5.5. QC Testing

- 5.5.1. Use the reserved aliquots for Hemoglobin Analysis test, QuantiChrom (IUGB-3-49).

6. REFERENCES

- 6.1. N/A

7. DOCUMENTATION

- 7.1. Master copies of SOPs are maintained by the IU Operations Manager.

8. APPENDICES

- 8.1. Appendix A: CSF Reference Pools

Aliquot volumes for CSF by pool

Project	Volume Size	Number of aliquots to create
PPMI PD Pool 1	250 μ L (for Hemoglobin QC)	3
	250 μ L	750
PPMI PD Pool 2	250 μ L (for Hemoglobin QC)	3
	250 μ L	550
PPMI Healthy Control Pool 1	250 μ L (for Hemoglobin QC)	3
	250 μ L	750
PPMI Healthy Control Pool 2	250 μ L (for Hemoglobin QC)	3
	250 μ L	550