Biological Fluid Collection & Handling
Standard Operating Procedure
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Faculty/ Department or Institute/Centre	Cumming School of Medicine, Department of Pediatrics, Neurocritical Care Program, ACH BioCORE	Date Created:	May 6 2016
		Created By:	Ming Qi
Location	*ACH B0-103*	Revision #:	1.2
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Supervisor	Dr. Michael Esser & Dr. David Sinasac	Revised By:	Matthew Rosin

Purpose

This document serves to provide guidance and step-by-step instructions for the collection, processing, handling, and storage of blood plasma, serum, buffy coat, cerebrospinal fluid (CSF), and urine samples. This SOP has been adapted from the Early Detection Research Network (EDRN) with the aim of biomarker discovery and validation [1].

Collection of biological specimens for clinical biomarker discovery mandates high quality specimens that have been prepared in a consistent manner. Variables in procedures for blood collection have been well documented as having impact in the outcome of clinical tests. These variables include i) the type of additive present in the blood collection tube, ii) sample processing times and temperature, iii) centrifugation force, iv) sample hemolysis, v) sample storage parameters (temperature, length of time), and vi) the number of freeze/thaw cycles [1-3]. Variation in sample handling can result in changes in protein stability and alteration in the quantity, compartmental distribution, chemical form, or decomposition state of small molecules in the sample. Further, systematic bias from inconsistency in practice can further confound research results and yield irreproducible data. The importance of this SOP is to ensure data reproducibility, utility, and interpretability.

Applicability

This document applies to all personnel of ACH BioCORE and can be applied to Clients of the BioCORE.

Authorization

Personnel must have completed WHMIS 2015 training, Biosafety (Bloodborne Pathogens) training, Biosafety (Biohazard Handling) training, Biosafety (Program) training, Hazard Assessment training, Laboratory Safety training, and Occupational Health and Safety training in the PeopleSoft Enterprise Learning program in the University of Calgary (or equivalent AHS training for BioCORE Clients).

Hazards associated with process

This procedure involves biosafety hazards related to handling biological samples (human blood and body fluids). See Authorization requirements that provide training for the proper handling of biosafety hazards.

Environment where task is to be undertaken

Personnel Name: _____ Date Printed: _____

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ACH lab in room B0-103 must be used for all steps in this procedure. The biological safety cabinet in room B0-111 (within lab B0-103) must be used for the relevant steps below.

Emergency Procedures

- If biohazardous liquid waste is spilled, it must be disinfected by 10 minutes of contact time with 10% bleach (if in a well ventilated area and if no personnel is working in this area during this time) or with DDAC 15%. Before beginning disinfection, notify all lab personnel in the area. Don PPE (lab coat, safety goggles, gloves), prepare fresh disinfectant, then treat the spill for 10 minutes before absorbing liquid with paper towel and placing it in a yellow biohazard waste container. Ensure the area is fully disinfected before continuing work.
- If the biohazardous liquid waste contacts your PPE, carefully discard contaminated PPE (gloves in yellow biohazardous waste, lab coat in soiled laundry for cleaning, and goggles can be disinfected using Virkon wipes) and don new PPE to continue work.

Safety Precautions

Personal Protective Equipment (PPE)	Engineering Controls
• Lab coat • Safety goggles • Nitrile gloves	• Use of Biosafety Cabinet (BSc) for all sample handling • Use of biohazard waste yellow containers • Use of UV lighting inside BSc for decontamination

Materials & Supplies

Item	BioCORE Recommendation	Details
Plasma tube	Lavender top: K_2EDTA spray-coated plastic tube (BD Vacutainer®)	• Universally more accepted than tubes containing citrate, heparin, sodium fluoride, or gels • Recommended for metabolite study
Serum tube	Red top: Silica spray-coated plastic tube (BD Vacutainer®)	• Tubes containing polymer gel (BD SST) have limitations of analytic incompatibility and polymer contamination of sample
CSF tube	15-mL screw-top, sterile (Falcon)	• Catalog #352096
Urine cup		

Personnel Name: _____ Date Printed: _____

0.5 mL vial	0.5 mL screw top sterile, Wheaton	• Catalog #W985874
1.2 mL vial	1.2 mL screw top, sterile, Wheaton	• Catalog #W985862
2 mL vial	2 mL screw top, sterile, Wheaton	• Catalog #W985852
4 mL vial	4 mL screw top, sterile, Wheaton	• Catalog #W985870
QR coded vial bottom insert	2D coded bottom insert for 0.5 mL vials, Wheaton	• Catalog #985881
Transfer pipettes	3.5 mL transfer pipettes, Sarstedt	• Catalog #86.1171
Pipettes	100-1000 uL 20-200 uL	• Sterile filter tips matching pipette sizes
Glass culture tubes	12 x 75 mm, sterile, VWR	• Catalog #
Markers	Black or blue	• Permanent, alcohol sensitive

Procedures

Plasma Preparation

1. Blood should be collected by venipuncture into a Lavender Top K_2EDTA vacutainer.
    - Volume should be filled to the calibrated volume of the tube for optimal blood-anticoagulant mixing ratio and sample consistency.
    - For pediatric patients: collection tube size should be selected according to draw volume.
2. Gently mix blood by inverting the tube 10 times at room temperature.
    - If not separated by centrifugation within 30 minutes, tubes should be refrigerated at 4C upright for no more than 4 hours.
3. Centrifuge the blood tube for 15 minutes at 1300 xg at room temperature.

4. During centrifugation, log new patient and/or new sample information into the FreezerWorks sample management database. Print off duplicate aliquot labels and affix 1 set to the paper documentation and the other set to aliquot vials.

5. Carefully transfer the top plasma layer (down to ~1 mm above buffy coat layer so as not to disturb buffy coat) into a glass culture tube using a transfer pipet. Combine plasma if using multiple tubes, and mix 10 times using transfer pipet to achieve sample homogeneity.

6. Aliquot sample evenly into a predesignated number of screw-top vials. Cap the vial tightly.

- Vials should be pre-labelled using a permanent marker with study ID, sample ID, date, and sample type.
- Vials should have a FreezerWorks unique barcode labels applied.

7. Place aliquots upright in a cryogenic sample storage box, properly label the box, and immediately place the box in a -80C BioCORE freezer.

- The estimated time period from venipuncture to sample storage in the freezer should be within 1 hour.
- The maximum time period should be within 5 hours.

<u>Buffy Coat Preparation</u>

1. Follow Plasma Preparation procedure steps 1-5.

2. After plasma is collected, use a new transfer pipet to collect all buffy coat, including some plasma above and coagulated blood below to ensure collection of all buffy coat.

3. Aliquot all buffy coat into a single screw-top vial. Cap the vial tightly.

- Pre-label vial using a permanent marker with study ID, sample ID, date, and sample type.
- Vial should have a FreezerWorks unique barcode labels applied.

4. Place aliquot upright in a cryogenic sample storage box, properly label the box, and immediately place the box in a -80C BioCORE freezer.

- The estimated time period from venipuncture to sample storage in the freezer should be within 1 hour.
- The maximum time period should be within 5 hours.

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Serum Preparation

1. Blood should be collected by venipuncture into a Red Top silica-coated vacutainer.
    - Volume should be filled to the calibrated volume of the tube for optimal blood-anticoagulant mixing ratio and sample consistency.
    - For pediatric patients: collection tube size should be selected according to draw volume.
2. Blood must to sit upright at room temperature for 30 minutes (maximum 60 minutes) to be allowed to clot.
    - If not separated by centrifugation after 60 minutes, tubes should be refrigerated at 4C upright for no more than 4 hours.
3. Centrifuge the blood tube for 15 minutes at 1300 xg at room temperature.
4. During centrifugation, log new patient and/or new sample information into the FreezerWorks sample management database. Print off duplicate aliquot labels and affix 1 set to the paper documentation and the other set to aliquot vials.
5. Carefully transfer the top plasma layer (down to ~1 mm above clot layer so as not to disturb clot) into a glass culture tube using a transfer pipet. Combine serum if using multiple tubes, and mix 10 times using transfer pipet to achieve sample homogeneity.
6. Aliquot sample evenly into a predesignated number of screw-top vials. Cap the vial tightly.
    - Vials should be pre-labelled using a permanent marker with study ID, sample ID, date, and sample type.
    - Vials should have a FreezerWorks unique barcode labels applied.
7. Place aliquots upright in a cryogenic sample storage box, properly label the box, and immediately place the box in a -80C BioCORE freezer.
    - The estimated time period from venipuncture to sample storage in the freezer should be within 1 hour.
    - The maximum time period should be within 5 hours.

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Urine Procedure

General Notes: Urine collections display high variability in the concentration of proteins and molecules. Common collection methods inclue i) 24-hour collection, ii) first morning urine, and iii) spot urine. Spot urine collection exhibits a higher degree of variation in concentration, but is more feasible for studies with many participants and additionally shows less proteolysis of the sample due to minimizing bladder retention time. Midstream collection of urine minimizes the problem of bacterial contamination. Although sometimes preservatives like sodium azide or boric acid are used for urine storage, these are not recommended due to lack of inter-lab consistency and the potential formation of chemical complexes in the sample. Current practice suggests urine collection methods dependent on the biomarker application. Whole urine analysis is beyond the scope of this SOP. The primary aim of the BioCORE is the analysis of metabolites by Mass Spectrometry and proteins and metabolites by ELISA/multi-plex. The following procedure is adapted from the protocols used by Human Kidney and Urine Proteomics Project [5], multiple cancer research institutes for biomarker discovery [6], and the EDRN [7]. The study subject should be informed to stay well hydrated on the date of urine collection. Our recommendation for urine collection method is to collect the second urine in the morning, which has an advantage of less variation compared to random spot collection.

1. Instruct patient (or guardian) to cleanse urethral area with castile soap towelette.

2. Collect midstream of the second morning urine, about 25-30 mL, in a graduated, sterile container.

3. Collection should be provided to research or clinical staff and placed on ice for immediate processing. If not immediately processed, collection can be put on ice for up to 20 minutes.

4. Pipette 25 mL raw urine into a sterile 50 mL conical tube with screw cap and cap tightly.

5. Centrifuge urine tube at 1500 xg for 10 minutes at 4C to pellet any cells and debris.

6. Decant the entire pool of soluble urine supernatant into a new labelled 50 mL conical tube.

7. Centrifuge the soluble urine at 10,000 xg for 30 minutes at 4C to reduce microbial load.

8. During this step, enter patient/sample info into FreezerWorks and print duplicate aliquot labels to affix to i) the sample paper record and ii) the aliquot vials. Note: enter sub-aliquots into FW and repeat.

9. Decant supernatant into a second new labelled 50 mL conical tube, cap, and vortex to mix.

10. Aliquot 5 mL of urine into 5 sterile 5 mL vials labelled with marker and FreezerWorks barcodes.

11. Sub-aliquot one of these 5 mL aliquots into 5 sterile 1mL vials marked and labelled.

12. Place vials upright in 3" or 2" labelled BioCORE freezer boxes and store in -80C freezer.

Cerebrospinal Fluid Procedure

General Notes: CSF collection must be performed by a licensed physician or experienced medical professional with consent from patient and/or guardian. CSF should be collected by lumbar puncture (LP) between the L3/L4 or L4/L5 lumbar vertebrae. LP should be performed between 8AM and 12PM following overnight fasting. The Sprotte type spinal needle with gauge 22G x 3½" should be used for the LP to minimize trauma and collection should yield clear fluid devoid of blood contamination. A matcher plasma or serum sample should also be collected prior to LP.

1. CSF from lumbar puncture is deposited into a sterile 15 mL conical tube with screw cap.

2. The minimum volume of CSF collection should be between 4-12 mL, which can be collected in serial into multiple tubes. Discard the initial mL if the fluid contains blood. Quality CSF should look clear.

3. Immediately centrifuge CSF at 2000 xg for 10 minutes at room temperature to pellet invisible blood components and cellular debris.

4. During this step, enter patient/sample info into FreezerWorks and print duplicate aliquot labels to affix to i) the sample paper record and ii) the aliquot vials.

5. Transfer supernatant to a labelled 15 mL conical tube. If multiple collections were obtained, CSF should be pooled, mixed by gentle inversion 5 times.

6. Aliquot 250 uL of CSF into sterile 0.5 mL vials labelled with marker and FreezerWorks barcodes.

7. Place the aliquots upright into a 2" BioCORE freezer box and store in -80C freezer.

- The optimal time period from CSF collection to freezing is under 2 hours.
- The maximum time period is 4 hours when CSF is kept at room temperature.

Patient & Sample Documentation Procedure

The minimum sample collection data required for entry into FreezerWorks in the BioCORE includes:

1. Study Name & PI
2. Study Identifier
3. Patient Demographics
4. Sample Identifier

Printed copies are UNCONTROLLED unless signed by an authorized BioCORE lab personnel below:

Personnel Name: _____ Date Printed: _____

5. Sample Type(s)
6. Sample collection details: Date, Time, Site, Volume,
7. Aliquoting requirements
8. Any deviation from Sample Collection SOPs
9. Additional comments about sample

References

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2. Bowen, R.A.R.; Remaley, A.T. Review: Interferences from blood collection tube components on clinical chemistry assays *Biochemia Medica* 2014; 24(1):31-34.

3. Bowen, R.A.R; Hortin, G.L.; Scako, G.; Otanez, O.H.; Remaley, A.T. Review: Impact of blood collection devices on clinical chemistry assays *Clin Biochem* 2010; 43:4-25.

4. CSF

5. Human Kidney & Urine Proteome Project: www.hkupp.org

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7. Early Detection Research Network: http://edrn.nci.nih.gov/resources/standard-operating-procedures/standard-operating-procedures/edrn-urine-collection-project-final-6-06-07-2.pdf