Unit Procedure

General Sample Processing

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Department: Processing Laboratory

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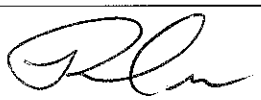
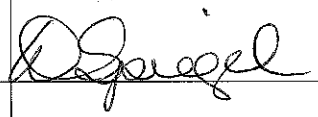
	Written by	Reviewed by	Approved by
Name	Robert Orr	Diana Spiegel	Christie Orschell
Job Title	CTSL Operations Manager	CRC Quality Assurance Manager	ATP Director
Signature			
Date	06 JAN 2017	6 Jan 2017	Jan 4, 2017

1. OBJECTIVE

This Standard Operating Procedure (SOP) defines the processes used to ensure that coagulated blood, anti-coagulated blood, buffy coat and urine is performed in a compliant and uniform manner at the Indiana Clinical and Translational Sciences Institute (CTSI) Clinical and Translational Support Laboratory (CTSL).

Processing variables may affect downstream analyses in unknown ways. Therefore, it is imperative that samples are processed uniformly even when protocol specific processing conditions are not defined. This SOP provides a recommended default set of processing variables. However, if processing is defined by a protocol and/or laboratory manual, this SOP defers to compliance with the protocol instuctions. The CTSI-CRC-PL-FM508 Lab Processing Sheet is utilized to document processing specifics including processing per this SOP or as directed by the protocol.

2. SCOPE

2.1. The SOP applies to CTSL and Clinical Research Center (CRC) personnel conducting general processing of research samples including blood and urine for the Indiana Clinical and Translational Sciences Institute (CTSI) CTSL. It is intended to provide basic procedures and parameters for processing blood and urine research specimens.

2.2. This processing SOP may be superseded by specific written directives from the investigator or sponsor as directed in CTSI-CRC-PL-151 Management of Requests for Sample Processing Support and/or the sponsor provided laboratory manual.

3. RESPONSIBILITIES

3.1. CTSL and CRC personnel are responsible for compliance with all procedures for processing research specimens defined by this SOP and/or by study provided instructions.

4. DEFINITIONS

CRC: Clinical Research Center	CTSI: Clinical and Translational Sciences Institute
CTSL: Clinical and Translational Support Laboratory	EDTA: Ethylenediaminetetraacetic acid
NaHep: Sodium Heparin	PI: Principal Investigator
PL: Processing Lab	RBC: Red Blood Cell
SOP: Standard Operating Procedure	SST: Serum Separator Tubes
UV: Ultraviolet Light	

5. ASSOCIATED DOCUMENTS

5.1. CTSI-CRC-QA-003 Document Control and Management
5.2. CTSI-CRC-CLN-030 Handling of SOP Deviations
5.3. CTSI-CRC-CLN-031 Handling of Protocol Deviations
5.4. CTSI-CRC-PL-121 General Safety
5.5. CTSI-CRC-PL-122 Radiation Safety Oversight
5.6. CTSI-CRC-PL-301 Mechanical Refrigeration Units
5.7. CTSI-CRC-PL-303 Centrifuges - Operation and Maintenance
5.8. CTSI-CRC-PL-160-01 Specimen Receipt, Tracking and Distribution
5.9. CTSI-CRC-PL-151 Management of Requests for Sample Processing Support
5.10. CTSI-CRC-PL-158 Data/Sample Management System

6. PROCEDURE

6.1. Reagents required –N/A
6.2. Supplies required:
  6.2.1. Cryovials
  6.2.2. Disposable Pipette(s)
  6.2.3. Cryovial screwcaps – the following color/sample matches are suggested only
    6.2.3.1. Red caps for serum samples
    6.2.3.2. Purple or green caps for plasma samples
    6.2.3.3. Blue caps for citrated samples
    6.2.3.4. Clear for buffy coat samples
  6.2.4. Wooden applicator sticks for blood clot retrieval.
6.3. Equipment required:
  6.3.1. Centrifuge - refrigerated
  6.3.2. 4°C/-20°C/-80°C Mechanical Refrigerators/Freezers
6.4. Light Sensitive Specimens
  6.4.1. Specimens that are identified by the protocol as sensitive to UV light must be processed under lighting conditions that have UV shielding in place.
    6.4.1.1. In rooms that have UV shielding in the overhead light fixtures, processing may be performed under biological safety cabinets that have shielding installed to protect samples from UV emanating from the internal light fixture.
    6.4.1.2. In rooms that do not have UV shielding in the overhead light fixtures, processing will be performed under biological safety cabinets that have been properly shielded from UV emanating from both the room lights and the internal light fixture.

6.5. Receive samples per CTSI-CRC-PL-160-01 Specimen Receipt, Tracking and Distribution

6.6. **Blood/urine sample centrifugation**

6.6.1. Set the centrifuge to the temperature/speed/duration specified in protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet or per Table 1 if protocol specific instructions are not provided. Refer to CTSI-CRC-PL-303 Centrifuges - Operation and Maintenance for instruction on setting and operating centrifuges.

6.6.2. Coagulated serum samples only - Ensure sample has clotted (coagulated) by inverting and observing that either the blood has reached a solid phase or that the required clot time has been reached per the protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet. If no clot is observed, contact study personnel for further directives.

6.6.3. Verify sample(s) is/are securely closed.

6.6.4. Place the draw tube(s) into the centrifuge such that the unit is centrifugally balanced. Utilize dummy/balance draw tubes to ensure balance if required.

6.6.5. Centrifuge the sample(s) and record centrifugation start time and centrifuge ID on protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet.

6.6.6. Remove the draw tube(s). Record centrifuge end time if requested.

6.7. **Coagulated blood sample processing**

6.7.1. There are 2 distinct layers (serum and clot containing red and white blood cells and platelets) in the tube (excluding the SST layer if applicable).

6.7.2. The serum layer is the layer on top, usually translucent.

6.7.3. Record notes on the protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet if the serum layer is hemolyzed (red tinged from lyses red blood cells), icteric (gold, brown or green colored), lipemic (cloudy) or any other observation as specified by the protocol to be noted.

6.7.4. Remove cap from tube

6.7.5. Remove the serum

6.7.5.1. Place a pipette tip directly into the serum and above the clot/red cells. Do not disturb or draw up into the tip any red blood cells. A small amount of serum may remain in the draw tube (approximately 100ul) as a result of not disturbing the RBC layer.

6.7.5.2. Aliquot the serum into cryovials per protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet until nearly all serum is recovered or all aliquots have been filled.

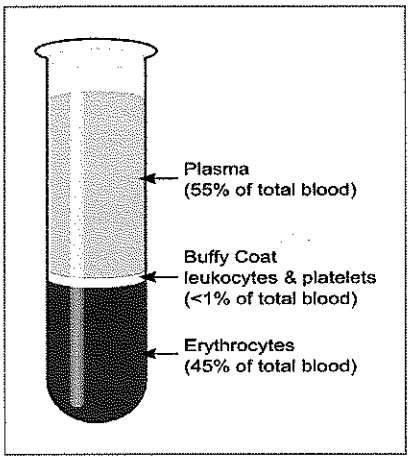
6.7.5.3. If the serum contains a significant fibrin (gel consistency) clot, it must be removed prior to aliquotting.

6.7.5.3.1. Insert 2 applicator sticks into the serum layer of the draw tube and twist to attach the fibrin to the sticks.

6.7.5.3.2. Press the fibrin clot against the side of the tube to expunge the serum and gently remove the clot to discard.
6.7.5.3.3. Record on protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet that fibrin clot was removed.
6.7.5.3.4. Remove sticks and repeat centrifugation.
6.7.5.4. Record on protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet the number of aliquots created.
6.7.6. Cap cryovial(s) using red cryovial screwcaps or any other color as requested per protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet.
6.7.7. If directed by protocol specific from CTSI-CRC-PL-FM508 Lab Processing Sheet, retain the clot and any free cells (RBCs).
6.7.7.1. Place a disposable pipette directly into the clot and move in a circular motion to loosen the clot from the tube.
6.7.7.2. Pour the cell clot and any free cells into a cryovial as specified in protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet or, if not specified, into an 8 mL cryovial.
6.7.7.3. Cap cryovial using a clear cryovial lid.
6.7.7.4. Record on protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet the number of aliquots created. Continue with step 6.12.

6.8. **Anti-coagulated blood sample processing**
6.8.1. There are three distinct layers (Plasma, Buffy Coat and Red Blood Cells) in the tube after centrifugation (see Fig. 1).
6.8.2. The plasma is the layer on top, usually translucent.
6.8.3. Record on protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet if the plasma layer is hemolyzed (red tinged from lysed red blood cells), icteric (gold, brown or green colored), lipemic (cloudy) or any other observation as specified by the protocol to be noted.

FIGURE 1



6.8.4. Remove cap from tube.
6.8.5. Remove the plasma
6.8.5.1. Place a pipette directly into the plasma being careful not to disturb the buffy coat and RBC layers.
6.8.5.2. Leave approximately a 1/8 inch of plasma on top of the buffy coat layer.
6.8.5.3. Aliquot the plasma into cryovials per protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet until all the plasma is removed or all aliquots have been filled.
6.8.6. Cap cryovials using the colored cryovial screwcaps as requested per protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet.
6.8.7. Perform additional processing as directed per protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet.
6.8.7.1. Buffy Layer Processing- if required per protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet, proceed to step 6.9
6.8.7.2. All other processing - follow as directed per protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet.
6.8.8. Discard buffy coat and red cells if no additional processes are required and continue with step 6.12.

6.9. **Buffy coat layer collection**
6.9.1. The buffy coat is the white layer of cells between the plasma and the red cells (see Figure 1)
6.9.2. Remove the buffy coat by placing the pipette tip directly on top of the buffy coat layer and draw it up while moving the tip in a circular motion until the entire buffy coat is removed (~0.5mL).
6.9.2.1. Minimize the amount of plasma and red cell collected with the buffy coat.
6.9.2.2. Aliquot buffy coat into 2ml cryovials unless otherwise specified by the protocol and/or protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet.
6.9.2.3. Close cryovials using clear cryovial screwcaps or any color cap as requested per protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet.
6.9.2.4. Discard red cells if no additional processes are required. Continue with step 6.12.

6.10. **Urine processing**
6.10.1. Measure the total volume of urine samples as requested per the protocol specific CTSI-CRC-PL-FM508 Lab Processing Sheet or protocol directives.

6.10.1.1. When possible, first obtain required aliquots of urine, being careful to accurately measure volume of each aliquot. Follow steps 6.10.3 through 6.10.5.
6.10.1.2. Calculate total volume of all aliquots.
6.10.1.3. Measure remaining urine sample using a clean and dry graduated cylinder.
6.10.1.4. Add total volume of aliquots to total volume of remaining sample and record result on the protocol specific CTSI-CRC-PL-FM508 Lab Processing Sheet
6.10.1.5. Record urine collection start date/time and stop date/time, if required, on the protocol specific CTSI-CRC-PL-FM508 Lab Processing Sheet
6.10.2. Whenever possible, pool urine that has been collected in multiple containers for the same collection time point prior to aliquotting and total volume measurement.
6.10.3. Using a disposable pipette or micropipette with disposable tip, aspirate urine from the collection container.
6.10.4. Aliquot the urine per protocol specific CTSI-CRC-PL-FM508 Lab Processing Sheet until all aliquots have been filled or sample is used up.
6.10.5. Cap cryovial(s) using yellow cryovial screwcaps or any other color as requested per protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet.
6.10.6. Perform additional processing of urine samples per protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet and proceed to step 6.12 for storage of aliquots.
6.10.7. Store original urine in their collection containers temporarily at 2-8˚C until all sample processing is completed. If requested by protocol, store urine temporarily at 2-8˚C until all sample analysis is completed.
6.10.8. When the urine is no longer needed, samples are disposed by flushing down the drain and collection containers rinsed with water prior to disposal.

6.11. **DNA/RNA Paxgene Tubes** – no processing required.
6.11.1. **DNA Paxgene tube** – unless otherwise instructed per protocol, store tube(s) per the following:
6.11.1.1. Place DNA Paxgene tube upright in a wire rack in a -20C freezer for 24 hours.
6.11.1.2. Relocate frozen tube to long term storage in a -70C freezer.
6.11.1.3. Record time in -20C, time of relocation to -70C and freezers that samples were stored in on the protocol specific CTSI-CRC-PL-FM508 Lab Processing Sheet
6.11.2. **RNA Paxgene tube** - unless otherwise instructed per protocol, store tube(s) per the following:
6.11.2.1. Store sample(s) upright at room temperature for a minimum of 2 hours and a maximum of 72 hours post draw time. Record time

placed upright on the protocol specific CTSI-CRC-PL-FM508 Lab Processing Sheet as "processing start time".

6.11.2.2. Store tube in a 70˚C freezer after step 6.11.2.1. is completed. It is preferred that the tube is frozen upright to prevent displacing the cap.

6.11.2.3. Record date and time tube is placed in freezer, along with freezer ID, on protocol specific CTSI-CRC-PL-FM508 Lab Processing Sheet.

6.11.2.4. If the sample was placed on ice or frozen prior to arrival at CTSL, note as such on the protocol specific CTSI-CRC-PL-FM508 Lab Processing Sheet. If the freeze time is available, enter provided time on protocol specific CTSI-CRC-PL-FM508 Lab Processing Sheet and attach any documentation that was received with sample confirming freeze time.

6.12. Store aliquoted or whole blood samples (other than Paxgene tubes – see 6.11 for Paxgene instructions) according to the protocol specific CTSI-CRC-PL-FM508 Lab Processing Sheet or per Table 1 if direction not provided by study.

6.13. Protocol deviations are managed per CTSI-CRC-CLN-031 Handling of Protocol Deviations.

6.14. SOP deviations are managed per CTSI-CRC-CLN-030 Handling of SOP Deviations.

6.15. Of special note, per scope of this SOP, complying with the special investigator specific directives that may or may not be prescribed on the protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet is not a deviation to this SOP but is noted on the applicable worksheet and documentation of the special directives is maintained in the appropriate study folder in the CTSL.

7. REFERENCES

7.1. Henry, John Bernard M.D., Clinical Diagnosis and Management by Laboratory Methods 19th Edition, 1996. Philadelphia, W.B. Saunders Company, 1996; 21:22

7.2. BD Vacutainer Blood and Urine Collection FAQs - www.bd.com

7.3. DNA Paxgene Tube freezing guidelines - http://www.preanalytix.com/sites/default/files/handbooks/PAXgene%20Blood%20DNA%20Tube%20Freezing%20Guidelines.pdf

7.4. RNA Paxgene Tube Product circular - http://www.preanalytix.com/sites/default/files/handbooks/PAXgene%20Blood%20RNA%20Tube%20IVD%20Product%20Circular.pdf

TABLE 1

Tube Type	Special Instructions	Centrifuge Settings			Storage
		Temp	Time (min)	Speed (g)	
Coagulated blood tubes: red, gold, tiger(red/black)	Allow to clot for a minimum of 30 minutes prior to start of centrifuge	Room Temp	10	1300	Keep ambient, refrigerate or freeze.
Anti-coagulated blood tubes: lavender, green	If there is a delay in processing, store tube in refrigerated temperatures.	Room Temp	10	1300	Refrigerate or freeze. Unprocessed tubes may be frozen as whole blood sample
Citrated blood tubes: blue	Keep refrigerated or on ice until processing start	Cold (~ 4°C)	15	1500	Refrigerate or freeze

8. APPENDICES

None

9. AMENDMENT HISTORY

Date of Amendment: NA

Amendment Request by: NA

Change Control No, if applicable: NA

Details of Amendment: NA