


Procedure for Serum Processing from Whole Blood

Title:	Procedure for Serum Processing of Whole Blood Collected in Clot (NON) or SST Tube (LDMS specimen code = BLD/NON/SER or BLD/SST/SER)		
Origination Date:	09 December 2011	Total Pages:	9
Effective Date:	05 February 2016	SOP Number	LTC-SOP-66 v1.0
Written By:	ACTG/IMPAACT Laboratory Technologist Committee (LTC)	Supersedes SOP Dated:	N/A

	Network	Name, Title	Signature	Date
Approved By (Network):	ACTG	Robert W. Coombs, MD, PhD, FRCPC ACTG Network Laboratory Principal Investigator		02FEB2016
	IMPAACT	Grace Aldrovandi, M.D. IMPAACT Network Laboratory Principal Investigator		

	Name, Title	Signature	Date
Reviewed By (Laboratory):			

	Version Effective Date (dd/mmm/yy)	Comments
Revision History		

Table of Contents

1	Purpose	3
2	Scope.....	3
3	Background	3
4	Authority and Responsibility.....	3
5	Biosafety	4
6	Sample Chain of Custody	4
7	Requirements.....	4
8	Equipment, Consumables and PPE	6
9	Procedure.....	7
10	Limitations	7
11	Literature References	7
12	Contributing Authors:	8
	<i>Appendix A: Example Supplies</i>	<i>9</i>

1 Purpose

The purpose of this SOP is to document the procedures for processing, storage, and shipment of serum specimens collected in blood collection tubes without anticoagulant and with or without serum separator gel.

2 Scope

ACTG and IMPAACT laboratories performing serum processing of whole blood collected in Clot or SST Tube.

This SOP is part of the ACTG/IMPAACT Laboratory Manual:

<https://www.hanc.info/labs/labresources/procedures/Pages/actgImpaactLabManual.aspx>

3 Background

Most protocols undertaken by the AIDS Clinical Trials Group (ACTG) and International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Group are clinical in nature, with both real-time and post-study requirements. Real-time assay results may be used to determine study participant responses to therapies and to determine when changes in protocol steps (i.e. changes in study participant management) are warranted. Post-study analyses are used to make recommendations for future treatment and management models for HIV-infected populations. Therefore, all specimens should be processed appropriately to ensure the best results for both individual study participant management and completion of the collective protocol objectives.

The success of a protocol depends upon the adequate collection, processing, storage, transport, and retrieval of specimens. Guidelines for sample collection and storage need to anticipate the requirements of future studies that are yet to be designed and technological advances which are in the early stages of development. While this is not always possible, certain basic tenets exist: for example, all specimens should be collected and processed using aseptic technique and universal safety precautions. This includes the use of sterile tubes, pipette tips and reagents, and a working environment designed to prevent contamination of samples and provide adequate safety measures for laboratory personnel.

4 Authority and Responsibility

- 4.1 The Network Laboratory Directors (or his/her designee) have the authority to establish, review and update this procedure.
- 4.2 The ACTG/IMPAACT Laboratory Technologist Committee (LTC) is responsible for the maintenance and control of SOP documentation.
- 4.3 The Laboratory Director is responsible for the implementation of this LTC SOP or laboratory-specific SOP, and for ensuring that all appropriate personnel are trained. A laboratory SOP must:

- 4.3.1 Include, without procedural modification, the portions of the current version of the LTC SOP that are used within the network site-affiliated laboratory
- 4.3.2 Reference the current version of the LTC SOP
- 4.4 All laboratory technicians are responsible for reading and understanding this SOP prior to performing the procedures described herein.
- 4.5 The site Principal Investigator and designees are responsible for understanding and adhering to the study participant preparation and specimen collection components.

5 Biosafety

It is essential that universal precautions be taken while working with whole blood. Appropriate personal protective equipment should be worn at all times to ensure safe handling of samples; this includes latex, or nitrile gloves, lab coats or gowns, and safety glasses where required. Torn gloves must be replaced immediately. In the event of a blood exposure or needle puncture, follow your site/institution policy for handling work-related injuries.

6 Sample Chain of Custody

- 6.1 Sample chain of custody is mandatory for all ACTG/IMPAACT specimens.
- 6.2 Verify that the specimens received match the clinical requisition and case report forms (CRFs). Notify the laboratory manager and clinic staff immediately if ANY discrepancies are noted so that appropriate attempts to recover specimens can be made.
- 6.3 It is essential that all protocol required primary specimens and all protocol required aliquots are logged into the Laboratory Data Management System (LDMS) in real time.
- 6.4 Aliquots received and/or stored on site must be logged into the LDMS storage module.

7 Requirements

- 7.1 Specimen Requirements:
 - 7.1.1 Refer to the protocol-specific Laboratory Processing Chart (LPC) to determine the tube type, volume of blood to be drawn, and quantity of specimen aliquots required for each collection.
 - 7.1.2 The most commonly used tube types for serum collection are NON Red top tube with no anticoagulant or SST (serum separator tube with clot activator and polymer separation gel for serum separation).
 - 7.1.3 There are several tube types that may be used for serum collection depending on the tube manufacturer and the analyte to be tested (for examples, see list below 7.1.4).

- 7.1.4 Blood collection tubes are available in:
 - 7.1.4.1 plastic or glass,
 - 7.1.4.2 with or without clot activator,
 - 7.1.4.3 with or without a thrombin-based clot activator,
 - 7.1.4.4 with or without silicone coating.
 - 7.1.4.5 Refer to BD Vacutainer® Venous Blood Collection Tube Guide for complete listing and description:

http://www.bd.com/vacutainer/pdfs/plus_plastic_tubes_wallchart_tubeguide_VS522_9.pdf

Note: Royal Blue additives are distinguished by the color of the label, not the stopper.

Note: Other tube types may be available from other manufacturers. Refer to the manufacturer's instructions.

- 7.1.5 Follow manufacturer's instructions to prevent hemolysis.
- 7.1.6 For unusual subject preparation or specimen collection requirements, refer to the protocol-specific LPC.
- 7.2 Specimen Management, Labeling and Shipping
 - 7.2.1 All stored specimens must be logged into the LDMS for specimen tracking, storage and shipping according to the LPC or other protocol-related documents. This includes all specimens destined for central testing or repository storage.
 - 7.2.2 Laboratories must use LDMS barcoded labels for their specimen aliquots. Aliquot labels must be LDMS-generated and must contain the following information:
 - 7.2.2.1 Global Specimen aliquot-unique ID # (Aliquot Specimen; only for specimen dates after September 1, 2005. Not required for hand-written specimens, collected prior to September 1, 2005)
 - 7.2.2.2 Protocol
 - 7.2.2.3 PID
 - 7.2.2.4 Specimen Date
 - 7.2.2.5 Visit Identifiers
 - 7.2.2.6 Primary/Additive/Derivative/SubAddDer

- 7.2.2.7 LDMS condition codes must be adjusted to document when modules are not available or otherwise do not meet protocol or network requirements, for example when specimens are lost, have not been processed or stored correctly, or have insufficient volume. Comments must be entered in the LDMS to explain the discrepancy with the requirements.
- 7.2.3 Aliquot storage locations and storage temperatures must also be recorded in the LDMS.
- 7.2.4 Laboratories are expected to export their data at least weekly to the Data Management Center (DMC). More frequent exporting is suggested for testing laboratories to assure timely distribution of assay results.
- 7.2.5 Specimen shipping must follow the protocol instructions, the [ACTG/IMPAACT Instructions for Overnight Shipments](#), the [ACTG/IMPAACT Specimen Repository Guidelines and any other ACTG/IMPAACT guidelines](#), SOPs or memos as applicable.

8 Equipment, Consumables and PPE

- 8.1 Laminar flow hood (minimum Class2, Type A biosafety cabinet)
- 8.2 Gloves (latex or nitrile)
- 8.3 Lab coat or protective gown
- 8.4 Centrifuge with horizontal swinging bucket rotor, with speeds up to 1800 x g and equipped with aerosol safe canisters
- 8.5 Sterile, plugged pipette tips; P200, P1000
- 8.6 Micropipettors; P200, P1000
- 8.7 Sterile Serological pipettes (10 mL, 5 mL volumes)
- 8.8 Pipette Aid
- 8.9 Sterile cryopreservation vials: 1.8 to 2mL with screw cap, external threads, and o-rings.

Note: Some cryovials are unacceptable for use in liquid nitrogen. Please check the manufacturer's recommendations before using. (example: Sarstedt cat #72.694.006 (flat), 72.693.005 (conical) or equivalent)

- 8.10 LDMS Cryo labels specific for use in freezing and liquid nitrogen- see FSTRF Portal and LDMS Barcode Implementation Messages
<https://www.fstrf.org/apps/cfm/apps/common/Portal/index.cfm>
-70° / -80°C freezer (range = -65 to -95 °C)

9 Procedure

- 9.1 Allow blood to clot for a minimum of 30, 40, or 60 minutes in a vertical position. Refer to protocol specific LPC for indicated clotting incubation times.
- 9.2 Observe a dense clot.
- 9.3 Centrifuge at room temperature (20°C to 25°C) at 1100 to 1300 x g for 10 minutes in swinging bucket rotor units or centrifuge at 1100 to 1300 x g for 15 minutes in fixed angle units.
- 9.4 SST tubes contain a polymer separation gel that will separate cellular clotted material from serum. After centrifugation, serum is located above the polymer barrier. Avoid the polymer barrier during pipetting.
- 9.5 Aliquot serum into sterile cryovials per protocol-specific instructions as described in the LPC.
- 9.6 Enter specimen information into LDMS including protocol number, PID number, week or visit value, draw time, draw date, processing start date, processing end date, and technician initials.
- 9.7 Print the expected number of LDMS labels required for the volume of serum processed and attach to serum aliquot vials.
- 9.8 Store serum aliquot vials between -65°C to -95°C.
- 9.9 If shipping blood collected in serum separator tube (SST), centrifuge as above, and send at ambient temperature (20-25°C). Serum (BLD/SST/SER) should be aliquoted and frozen within 30 hours of collection.

10 Limitations

Serum testing may be impacted by the presence of interfering substances such as lipemia and hemolysis. Fasting specimens must be collected according to protocol instructions. Rejection criteria must be determined by the testing laboratory.

11 Literature References

- 11.1 BD Vacutainer® Venous Blood Collection Tube Guide:
http://www.bd.com/vacutainer/pdfs/plus_plastic_tubes_wallchart_tubeguide_VS5229.pdf
- 11.2 How to Prepare a Quality Sample Using BD Vacutainer® SST™ Tubes:
http://bd.com/vacutainer/pdfs/plus_plastic_tubes_instructions_SSTsample_VS5177.pdf
- 11.3 Quest Diagnostics Test Center:
<http://www.questdiagnostics.com/hcp/qlim/testMenuSearch.do>

- 11.4 Blood Collection, A Short Course, 2nd edition; F.A. Davis Company, Philadelphia. PA, 19103; 2010.

12 Contributing Authors:

- 12.1 Patricia Anthony, Laboratory Manager, University of Southern California
- 12.2 Christopher Lane, Director, Clinical Trials Processing Laboratory, University of Rochester, New York.

Appendix A: Example Supplies

Reagent/Supply	Example(s)
Marking pens	Fisher Scientific Fisherbrand Marking Pens cat#13-379, or Nalgene® Lab Pen/Lab Marker #6310/#6311, or equivalent
Absorbent Material	Saf-T-Pak STP-151, or equivalent
Cryotube externally threaded with O-ring	Corning® 2mL external thread polypropylene cryogenic vial, self-standing with round bottom #430659 Nunc CryoTubes™, external thread, polypropylene (PP) tubes and screwcap #377267 WHEATON Cryule® Plastic Cryogenic Vials, external thread, #985742 SARSTEDT Screw cap micro tube, external thread, #72.694.006
Platform Shaker	Cole-Parmer Economical Orbital Shaker; 16" x 16" Platform, 120 VAC, EW-51820-40, or equivalent.