Purpose

The purpose of this SOP is to describe the standard procedures involved in processing and storing of study blood (EDTA and Red Top) samples after the sample has been delivered to the laboratory.

Responsibility

This SOP applies to any study laboratory staff. It is the responsibility of those users to follow the guidelines stipulated herein.

The Principal Investigator (through the study coordinator when applicable) retains the overall responsibility of implementation of these standard procedures.

The study laboratory coordinator is responsible for answering questions you may have about the content of this SOP and any other relevant study documentation. Please contact that the study laboratory coordinator through your site coordinator.

Abbreviations/Definitions

EDTA Ethylene Diamine Tetra acetic Acid
CRF Case Record Form
CBC Complete Blood Count
SOP Standard Operating Procedure
RPM Revolutions per Minute

Materials

1. EDTA pink tops (2 mls)
2. Red top serum tubes (2 ml)
3. EDTA pink tops (500 µl)
4. Sample storage vials – Nunc 2 ml cryotubes
5. Pipettes 200 µl and 1ml
6. Pipette tips 200 µl and 1ml tips
7. -80 freezer
8. Temperature controlled centrifuge machine
9. Nalgene cryobox System 100 (10 x 10 boxes)

Methods

1.0 General considerations

1.1 Samples collected from patients in this study will be for study-specific analyses.

1.2 Correct specimen collection bottles and correct request forms must always be used and verified at each collection.

1.3 Ensure all samples should be labelled with the Country code, site code, collection time-point code, (see Site Specific Collection Schedule (appendix 7.2)), specimen type, patient and date of collection. For example: **10-001-A0-W-XXX-12/10/2014**. For sample type, P= plasma from EDTA tube, S=serum from red top tube and W=whole blood from 500 µl EDTA tube.

1.4 Keep samples on ice, with ice packs at all times.

1.5 For EDTA and serum blood, if limited amount of sample, P1/S1 have priority.

1.6 There should be a minimum of 200 µl of sample per aliquot before introducing a new aliquot. For example, if there is 300 µl of EDTA plasma, put 200 µl in P1 and 100 µl in P2. If sufficient sample divide into two even aliquots.

1.7 Store each aliquot in separate 2 inch high Nalgene system 100 plastic freezer boxes. The idea is that sample aliquots go to specific analytic sites for the specific analyses and are separated at this stage to facilitate an efficient pre-transportation process.

1.8 Each freezer box should be labeled on the top and on the side. The label should contain a unique number letter combination (see sample freezer box storage log – Appendix 7.4).

1.9 Purposes of the samples are for later investigation on biochemistry, immune and metabolic markers.

1.10 Gloves must be worn at all times when handling specimens. This includes during removal of the rubber stopper from the blood tubes, centrifugation, pipetting, disposal of contaminated tubes, and cleanup of any spills. Tubes, needles, and pipets must be properly disposed of in biohazard containers, in accordance with institutional requirements.

1.11 The time between arrival at the laboratory and freezing (dry ice, liquid nitrogen or -80 ^{o}C freezer storage) should be maximally 60 minutes. This will be monitored very closely for every site and any deviation on sample transportation and processing time will be communicated. Prolonged delays of sample storage will compromise the integrity of the sample affecting the quality of lab results and will not be included in data analysis. Temporary storage at -20 ^{o}C is not allowed.

2.0 EDTA Sample Processing (2ml EDTA and 500 ul EDTA)

2.1 Transfer the whole blood in the 500 µl EDTA tube into a cryovial and store at -80^{o} Celcius in the “W” box after barcode labeling without additional processing.

2.2 Cross check the sample collection time point against the site specific Sample Collection Schedule. If the Sample Collection Schedule indicates that a clinical CBC is due and you have two 2 ml EDTA tubes, make an aliquot of 500 µl sample for CBC analysis according to local protocols. The remaining 1.5 ml of the EDTA sample should undergo the following procedure.

2.3 Centrifuge 2 ml EDTA sample (not whole blood 500 µl EDTA sample) at ~100g / 2200-2500 RPM (depends on centrifuge diameter) for 10 min at 6 degrees Celcius to obtain plasma.

2.4 Divide the centrifuged sample into two even aliquots and transfer it to two cryovials (P1 and P2).

2.5 Transfer the blood from the non-centrifuged 500 µl EDTA sample to one cryovial (W). If there is only the 2 ml EDTA, after centrifugation and removal of plasma, transfer the remainder to one cryovial (W).

2.6 Be very careful not to pick up red blood cells when aliquoting. This can be done by keeping the pipet above the red blood cell layer and leaving a small amount of plasma in the tube.

2.7 Add red sticker to vials for samples from subjects who have not consented to international shipping and store them at your site in a designated cryobox clearly labelled i.e. “Whole blood not for shipment”.

2.8 Store aliquots in -80^{o} Celsius freezer.

2.9 Put the samples in the polycarbonate freezer box (Nalgene 100 system) and put in the -80 freezer. Again each sample aliquot will go into a separate freezer box for that particular type of aliquot. This means all P1 samples go into the P1 box, P2 into the P2 box.

3.0 Serum Sample Processing

3.1 Cross check the sample collection time point against the Site Specific Sample collection Schedule (appendix 7.3). If two serum bottles are received and the Sample collection

Schedule indicates that a biochemistry is due, use the smaller volume sample for biochemical analysis according to local protocols. The larger volume sample should undergo the following procedure.

3.2 Serum Samples (red top tubes) should be spun after blood has completely clotted, i.e., after ~30 minutes of collection from the patient.

3.3 Centrifuge samples at ~100g/ 2200-2500 RPM (depends on centrifuge diameter) for 10 min at 4 degrees Celsius.

3.4 Centrifuged serum should be dived evenly into 2 even aliquots and transferred to cryovials (S1 and S2).

3.5 Be very careful not to pick up red blood cells when aliquoting. This can be done by keeping the pipet above the red blood cell layer and leaving a small amount of serum in the tube.

3.6 Add red sticker to vials for samples from subjects who have not consented to international shipping and store them at your site in a designated cryobox clearly labelled i.e. “Serum not for shipment”.

3.7 Put the samples in the polycarbonate freezer box (Nalgene 100 system) and put in the -80 freezer. Again each sample aliquot will go into a separate freezer box for that particular type of aliquot. This means all S1 samples go into the S1 box, S2 into the S2 box etc.

4.0 Sample log and registration

4.1 At the laboratory where samples are being processed and divided into aliquots, the Sample shipment log MUST be filled out.

4.2 Record time of receiving of sample and freezing of samples on the Sample Shipment Log.

4.3 Record in the log if less than the optimal amount of sample is stored (see appendix 7.2) and document the amount of volume stored as specific aliquots.

4.1 Document history

Version 1	Author	Approved by	Dated	SOP No:
1.03 **CHAIN Blood sample processing**	Robert Bandsman	Caroline Tigoi	10/11/2016	**CHN36**
1.04 **CHAIN Blood sample processing**	Robert Bandsman	Caroline Tigoi	06/01/2017	**CHN36**

5.0 Site training record

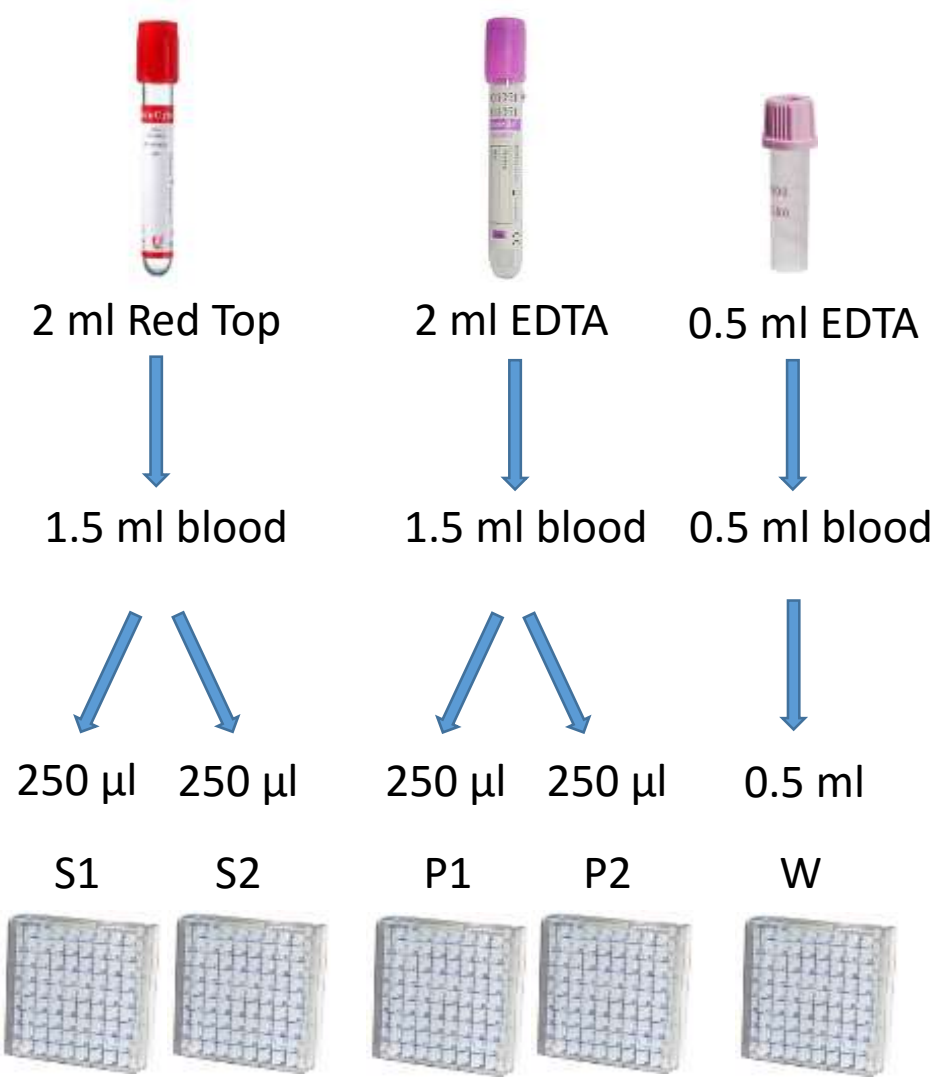
All sites are required to maintain a master copy of this SOP that documents the site staff that have been trained on this SOP.

Document History				
Version No.	**Trained staff initials**	**Signature of trained staff**	**Date**	**Trainer’s Initials**
1.01	KDT	Example row	1st Jan 2016	DM

6.0 References

7.0 Appendices

Appendix 7.1: Diagram Sample Processing



SITE NAME:		STUDY NAME:			POINT OF ORIGIN:	
DESTINATION:		PI NAME:			DATE:	
Subject ID	Specimen Type*	Specimen ID (Barcode number)	Visit No**	Date Collected	Time collected	Comments

Appendix 7.2 Sample Shipment Log

SHIPPED BY_____**DATE**_____ **(DD/MM/YYYY) TIME**_____ **TEMP:** _____

RECEIVING _____**DATE**_____ **(DD/MM/YYYY) TIME**_____**TEMP:**_____

STORED BY_____**DATE**_____ **(DD/MM/YYYY) TIME**_____

KEY
Visit Numbers:**
Visit Numbers: A0**-Admission; **A2**- Day 2; **A5** - Day 5; **D0**-Discharge; **D1**-Day 45; **D2** - Day 90, **D3** - Day 180, **RA** – Readmission **AD**- Deterioration and **CP**- Community participant

Specimen Type*: Stool (F1, F2, and F3), Blood (Whole blood, Plasma, Serum or DBS) or Rectal Swab (R1and R2)

Appendix 7.3 Site Specific Collection Schedule

Site Specific Sample collection Schedule

Migori

Tube	Volumes									
	Admission	D 2	D 5	Discharge	D 45	D 90	D 180	Readmission	Deterioration	Community participant
Time point code	AO	A2	A5	D0	D1	D2	D3	RA	AD	CP
EDTA 1 (Purple)	0.5 ml			0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml		0.5 ml
EDTA 1 (Purple)	1.5 ml	1.5 ml	1.5 ml	1.5 ml				1.5 ml	0.5 ml	1.5 ml
Serum 1 (Red)	0.5ml			0.5ml				0.5ml		
Serum 1 (Red)	1.5 ml			1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml		1.5 ml
DBS	1			1				1		1
Blood glucose	1							1	1	
HIV RDT	1									1
Malaria RDT	1							1	1	
Rectal swabs	2			2	2	2	2	2		2
Whole stool	1			1	1	1	1	1		1
Dual sugar test				If selected						
Urine				1			1			1

Tube	Volumes									
	Admission	D 2	D 5	Discharge	D 45	D 90	D 180	Readmission	Deterioration	Community participant
Time point code	AO	A2	A5	D0	D1	D2	D3	RA	AD	CP
EDTA 1 (Purple)	0.5 ml			0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
EDTA 1 (Purple)	1.5 ml			1.5 ml				1.5 ml	0.5 ml	1.5 ml
Serum 1 (Red)	0.5 ml			0.5 ml				0.5 ml		
Serum 1 (Red)	1.5 ml			1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml		1.5 ml
Blood culture	2 ml							2 ml	2 ml	
DBS	1			1				1		1
Blood glucose	1							1		
HIV RDT	1								1	1
Malaria RDT	1							1	1	
Rectal swabs	2			2	2	2	2	2		2
Whole stool	1			1	1	1	1	1		1

Tube	Volumes									
	Admission	D 2	D 5	Discharge	D 45	D 90	D 180	Readmission	Deterioration	Community participant
Time point code	AO	A2	A5	D0	D1	D2	D3	RA	AD	CP
EDTA 1 (Purple)	0.5 ml			0.5 ml	0.5	0.5	0.5	0.5 ml		0.5 ml
EDTA 1 (Purple)	1.5 ml			1.5 ml				1.5 ml	0.5 ml	1.5 ml
Gas/lactate	0.14 ml	0.14 ml	0.14 ml	0.14 ml					0.14	
Blood Culture	2 ml							2 ml	2 ml	
Serum 1 (Red)	0.5ml			0.5ml				0.5ml		
Serum 1 (Red)	1.5 ml			1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml		1.5 ml
Sodium Heparin (Green)		2 ml		2 ml	2 ml	2 ml	2 ml			2ml
DBS	1			1						1
Blood glucose	1							1		
HIV RDT	1									1
Malaria RDT	1							1	1	
Rectal swabs	2			2	2	2	2	2		2
Whole stool	1			1	1	1	1	1		1

Tube	Volumes									
	Admission	D 2	D 5	Discharge	D 45	D 90	D 180	Readmission	Deterioration	Community participant
Time point code	AO	A2	A5	D0	D1	D2	D3	RA	AD	CP
Samples sent to Dr. Joloba's laboratory										
EDTA 1 (Purple)	0.5 ml			0.5 ml			0.5 ml	0.5 ml		0.5 ml
EDTA 2 (Purple)	1.5 ml			1.5 ml			1.5 ml	1.5 ml		1.5 ml
Serum 1 (Red)	1.5 ml			1.5 ml			1.5 ml	1.5 ml		1.5 ml
DBS	1			1			1	1		1
Rectal swabs	2			2	2	2	2	2		2
Whole stool	1			1	1	1	1	1		1
Point of Care Testing										
Blood glucose	1							1	1	
HIV RDT	1									1
Malaria RDT	1							1		1
Samples sent to CORE lab										
CBC with diff	0.5 ml	0.5 ml		0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Chemistries	1 ml			1 ml				1 ml	1 ml	1 ml
Samples below sent to JCRC Immunology lab										
Sodium Heparin (Green)		1.5 ml		1.5 ml*	1.5 ml	1.5 ml	1.5 ml*			1.5 ml
CPT (Blue/black)		4 ml max		4 ml max*	4 ml max	4 ml max	4 ml max*			4 ml max
Urine		Up to 4 ml**			Up to 4 ml‡		Up to 4 ml‡			
For children eligible for TB sub study only—Joloba TB lab										
Induced sputum			Send X 1 during hospitalization					Send X 1 during hospitalization		
Whole stool			Send X 1 during hospitalization					Send X 1 during hospitalization		

*may be deferred so that total volume of blood for research does not exceed 1 ml/kg. Do not collect on Friday, Saturday, or Sunday

**for children eligible for TB sub-study only

‡for children on active TB treatment only

Tube	Volumes									
	Admission	D 2	D 5	Discharge	D 45	D 90	D 180	Readmission	Deterioration	Community participant
Time point code	AO	A2	A5	D0	D1	D2	D3	RA	AD	CP
EDTA 1 (Purple) CBC with diff	0.5 ml	0.5 ml		0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
EDTA 1 (Purple)	1.5 ml	1.5 ml		1.5 ml				1.5 ml	1.5 ml	1.5 ml
Blood culture*	2ml	2ml						2ml	2ml	
Serum 1 (Red)	2.0 ml	2.0 ml		2.0 ml	1.5 ml	1.5 ml	1.5 ml	2.0 ml	0.5 ml	1.5 ml
DBS	1	1		1				1		1
Blood glucose	1	1						1		
HIV RDT	1									1
Rectal swabs	2	2		2	2	2	2	2		2
Whole stool	1	1		1	1	1	1	1		1
Malaria RDT	1							1		

*only if clinically indicated (i.e. if child has symptoms of sepsis)

Tube	Volumes									
	Admission	D 2	D 5	Discharge	D 45	D 90	D 180	Readmission	Deterioration	Community participant
Time point code	AO	A2	A5	D0	D1	D2	D3	RA	AD	CP
EDTA 1 (Purple)	0.5 ml			0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
EDTA 1 (Purple)	1.5 ml			1.5 ml				1.5 ml	0.5 ml	1.5 ml
Gas/lactate	0.14 ml	0.14 ml	0.14 ml	0.14 ml					0.14	
Blood culture	2ml							2ml	2ml	
Serum 1 (Red)	0.5 ml			0.5ml			0.5ml	0.5ml		0.5ml
Serum 1 (Red)	1.5	1.5		1.5			1.5	1.5		1.5
DBS	1			1						1
Blood glucose	1							1		
Blood gas	0.1 ml	0.1 ml	0.1 ml	0.1 ml				0.1 ml		
HIV RDT	1									1
Rectal swabs	2			2	2	2	2	2		2
Whole stool	1			1	1	1	1	1		1
Dual sugar test				If selected						
Malaria smear	1							1		1
Urine storage				1			1			1
	TB SUB-Study									
Gastric/ Bronchial Aspirate	1									
Stool Gene xpert	1									
Urine storage	1					1	1			

Tube	Volumes									
	Admission	D 2	D 5	Discharge	D 45	D 90	D 180	Readmission	Deterioration	Community participant
Timepoint code	AO	A2	A5	D0	D1	D2	D3	RA	AD	CP
EDTA 1 (Purple)	0.5 ml			0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
EDTA 1 (Purple)	1.5 ml			1.5 ml				1.5 ml	0.5 ml	1.5 ml
Serum 1 (Red)	0.5 ml			0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	
Serum 1 (Red)	1.5ml			1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml		1.5 ml
DBS	1			1						1
Blood culture	1							1	1	
Blood glucose	1							1	1	
HIV RDT	1									1
Rectal swabs	2			2	2	2	2	2		2
Whole stool	1			1	1	1	1	1		1
Malaria RDT	1							1		

Tube	Volumes									
	Admission	D 2	D 5	Discharge	D 45	D 90	D 180	Readmission	Deterioration	Community participant
Timepoint code	AO	A2	A5	D0	D1	D2	D3	RA	AD	CP
EDTA 1 (Purple)	0.5 ml			0.5 ml				0.5 ml		0.5 ml
EDTA 1 (Purple)	1.5 ml			1.5 ml				1.5 ml	0.5 ml	1.5 ml
Serum 1 (Red)	0.5ml			0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	
Serum 1 (Red)	1.5 ml			1.5 ml	1.5 ml	1.5ml	1.5 ml	1.5 ml		1.5ml
DBS	1			1						1
Blood glucose	1							1	1	
Blood culture	1							1		
Blood gas	0.1 ml	0.1 ml	0.1 ml	0.1 ml				0.1 ml		
HIV RDT	1									1
Rectal swabs	2			2	2	2	2	2		2
Whole stool	1			1	1	1	1	1		1
Malaria RDT	1							1		

Appendix 7.4: Sample freezer box storage log

CHAIN Whole Blood samples Box 1	A	B	C	D	E	F	G	H	I
1	1-1-1740 124322 02/09/2016	1-1-1741 126234 02/09/2016	1-1-1743 122309 02/09/2016	1-1-1744 126241 02/09/2016	1-1-1745 126242 02/09/2016	1-1-1748 123838 03/09/2016	1-1-1749 125523 03/09/2016	1-1-1747 126258 04/09/2016	1-1-1750 126259 04/09/2016
2	1-1-1752 126263 04/09/2016	1-1-1753 125133 04/09/2016	1-1-1751 121934 05/09/2016	1-1-1754 126267 05/09/2016	1-1-1756 126273 05/09/2016	1-1-1757 126271 05/09/2016	1-1-1755 123610 05/09/2016	1-1-1760 126274 05/09/2016	1-1-1758 126287 06/09/2016
3	1-1-1769 125887 06/09/2016	1-1-1770 125283 06/09/2016	1-1-1762 126279 07/09/2016	1-1-1763 124053 07/09/2016	1-1-1765 122460 07/09/2016	1-1-1764 124224 07/09/2016	1-1-1759 125318 08/09/2016	1-1-1767 125766 08/09/2016	1-1-1768 125755 08/09/2016
4	1-1-1775 125876 08/09/2016	1-1-1771 126320 08/09/2016	1-1-1772 126322 08/09/2016	1-1-1773 126323 09/09/2016	1-1-1774 126351 09/09/2016	1-1-1776 126319 09/09/2016	1-1-1777 125860 09/09/2016	1-1-1778 126321 09/09/2016	1-1-1780 126233 09/09/2016
5	1-1-1766 125474 10/09/2016	1-1-1783 123904 10/09/2016	1-1-1784 126412 10/09/2016	1-1-1785 126409 10/09/2016	1-1-1786 126415 11/09/2016	1-1-1787 124932 11/09/2016	1-1-1788 123644 11/09/2016	1-1-1789 126416 11/09/2016	1-1-1790 126417 11/09/2016
6									
7									
8									
9									