

CHAIN BLOOD SPOT COLLECTION SOP (MASTER)



CHN 32: CHAIN Blood Spot Collection SOP (MASTER)

Purpose

The purpose of this SOP is to describe the standard procedures involved in collection and transport to the laboratory of study blood **spot** samples. This SOP applies inpatients and community participants.

Responsibility

This SOP applies to nursing staff, study clinicians and fieldworkers of study sites who will be undertaking the collection of blood spot samples. It is the responsibility of the 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

DBS	Dry blood spot
SOP	Standard Operating Procedures
CRF	Case Report Form

Required material

- CRF appropriate to time point
- Site Specific Sample Collection Schedule
- Study Sample Collection Log
- Warman 903 filter paper spot cards
- Disposable gloves
- Alcohol swabs, isopropyl alcohol/ spirit
- Cotton balls/ dry swabs
- Ziplock bags
- Whatman™ 10548234 Desiccant Packs for 903 Protein Saver Cards/Silica Gel desiccant

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Methods

1.0 General considerations

- 1.1 Samples collected from patients are study and site specific.
- 1.2 The admission sample should ideally be taken within 30 minutes of the patient being assessed in the emergency department and deemed eligible for the study, however if this is not possible due to emergency treatment the sample should be taken within 30 minutes after the child has been cleared for transport to the ward.
- 1.3 Blood draws can be performed by the clinic/research nurse, clinical officer or clinician. All research blood draws should ideally be timed with clinical blood draws where possible to reduce the number of venepunctures to the child.
- 1.4 Universal precautions and Occupational Safety and Health Administration and institutional requirements (<http://www.osha.gov/SLTC/biologicalagents/index.html>) should be followed.
- 1.5 If any study staff are pricked by a used needle or are otherwise concerned that they have been exposed to blood borne pathogens they should review the post-exposure prophylaxis SOP (CHN30) in addition to the Institutional Infection Control Policy.

2.0 Blood draw

- 2.1 Venepuncture and blood draw should be done in a procedure room when possible.
- 2.2 The phlebotomist should wear disposable gloves and use aseptic technique during phlebotomy.
- 2.3 New sterile, single use needles, are to be used for each blood draw, and after completion needles must be properly disposed of in a puncture resistant container.
- 2.4 Verify that this is the correct participant.
- 2.5 Explain the blood drawing procedure to the family and patient and reassure them that it is a safe procedure, but it may cause some distress.
- 2.6 Wash hands with soap and water
- 2.7 Palpate and choose a vein. The preferred sites for phlebotomy are the median antecubital veins of the upper extremity. Veins on the dorsum of the hand and other forearm veins are possible alternative sites. A tourniquet may be used to transiently distend veins prior to drawing blood. Do not leave the tourniquet too long.
- 2.8 Femoral vein blood draw is not advised unless being used for clinical bloods.
- 2.9 Disinfect the phlebotomy site by swabbing the skin in small outward circles with an alcohol swab. Do not touch the prepared puncture site with your fingers after disinfecting the skin.
- 2.10 Using aseptic technique, insert the needle of the vacutainer device into the vein.
- 2.11 Alternatively, you may perform a finger or heel prick to obtain the blood spot. The finger or heel prick technique is advised from admission when blood glucose and HIV testing are required. For advice on the finger/heel prick technique see the blood glucose monitoring SOP (CHN 34). It is also acceptable to remove a minimal amount of 50 to 80 μ l of blood from a red top serum tube to create a blood spot using a pipette and a filter tip (figure 3).
- 2.12 If heel prick is used, while holding the foot correctly, apply and release pressure to allow a drop of blood to form. Do not squeeze or “milk” the puncture site as this may dilute the blood with tissue fluid.
- 2.13 Allow a large drop of blood to collect. Once a drop of blood has formed, lightly touch the drop to the pre-printed circle on the filter paper allowing it to soak onto the circle. Allow the



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- next drop of blood to form and soak it onto the next marked circle (see figure 1 and 2).
- 2.14 Repeat until all three marked circles are adequately filled with blood. The pre-printed circles hold 70-80 µl blood each when fully filled. The size of the blood spot and the penetration of the spot through to the reverse side of the card allow for some assessment of the blood volume. The blood drop must have formed a clear circle at the back of the filter paper in order to have sufficient material.
 - 2.15 Do not excessively saturate the card with blood. Do not touch or attempt to smear the blood spots.
 - 2.16 Hold the Whatman Protein Saver 903 Card without touching the filter paper using powder free gloves.
 - 2.17 Filter paper card should be labelled with a barcode label containing the following variables; the Country code, site code, collection time-point, (see Site Specific Collection Schedule (appendix 7.2)), specimen type, Participant ID and date of collection. For example: **10-001-A0-DBS-XXX-12/10/2014**.
 - 2.18 Let the paper dry for 15 minutes on a Whatman Protein Saver 903 Card/or any other common drying rack (Figure 4), placed on clean and flat surface and then place in zip lock bag with a desiccant and seal bag.
 - 2.19 Samples should be transported and arrive at the laboratory within 30 minutes after collection.
 - 2.20 The DBS should be stored in -80°C freezer within 30 minutes of arrival in the laboratory.
 - 2.21 Store a maximum of 20 DBS cards in one cryobox and label the box at the top and on the side with the barcode numbers of the first and the last DBS card contained in the box.

3.0 Sample log and registration

- 3.1 All samples collected from a participant MUST first be logged in the Study Sample shipment log and the appropriate CRF, available in the ward/ study office.
- 3.2 Record time of collection on the Sample shipment log.

4.0 References

1. http://www.unicef.org/southafrica/SAF_resources_pcrtesting.pdf Toto Bora Blood
2. <http://www.spotonsciences.com/wp-content/uploads/AACC-Filter-paper-Poster-2013.pdf>
3. Sharma et al., Dried blood spots: Concepts, present status, and future perspectives in bioanalysis. Drug Testing and Analyses. 2014

5.0 Document history

Version	Author	Approved by	Dated
1.03 CHAIN BLOOD SPOT COLLECTION SOP (MASTER) CHN 28	Robert Bandsma	Caroline Tigoï	10/11/2016
1.04 CHAIN BLOOD SPOT COLLECTION SOP (MASTER) CHN 28	Robert Bandsma	Caroline Tigoï	06/01/2017

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6.0 Site training record

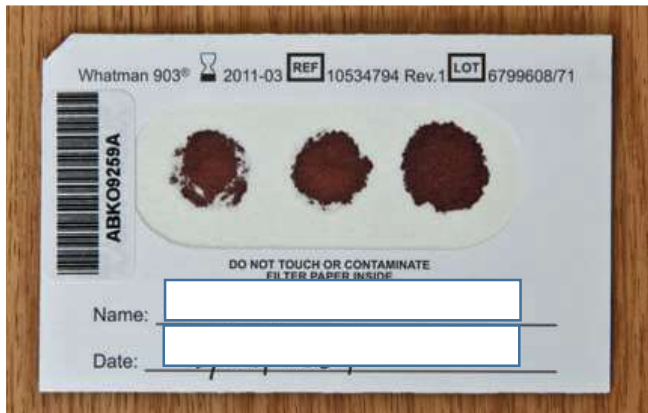
All sites are required to maintain a master copy of this SOP that documents the site staff that have been trained on this SOP.

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

Figure 1. Front Blood Spot Card



Figure 2. Back Blood Spot Card



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Figure 3: Pick 50 to 60 μ l of blood from the red top vacutainer using a pipette and drop it on the DBS spot

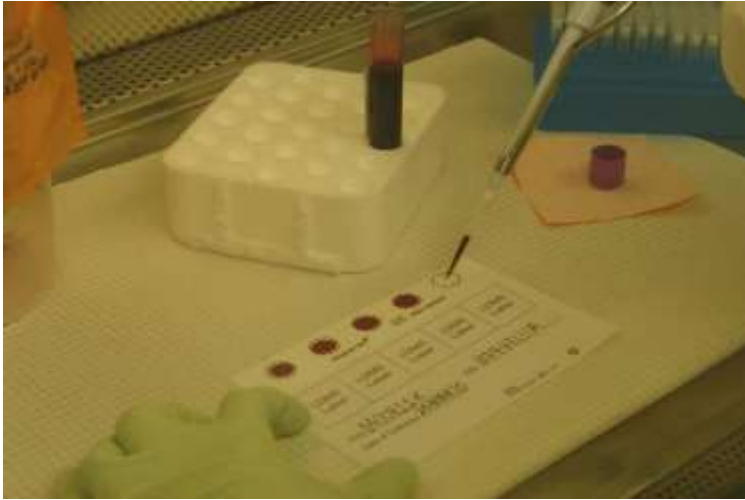
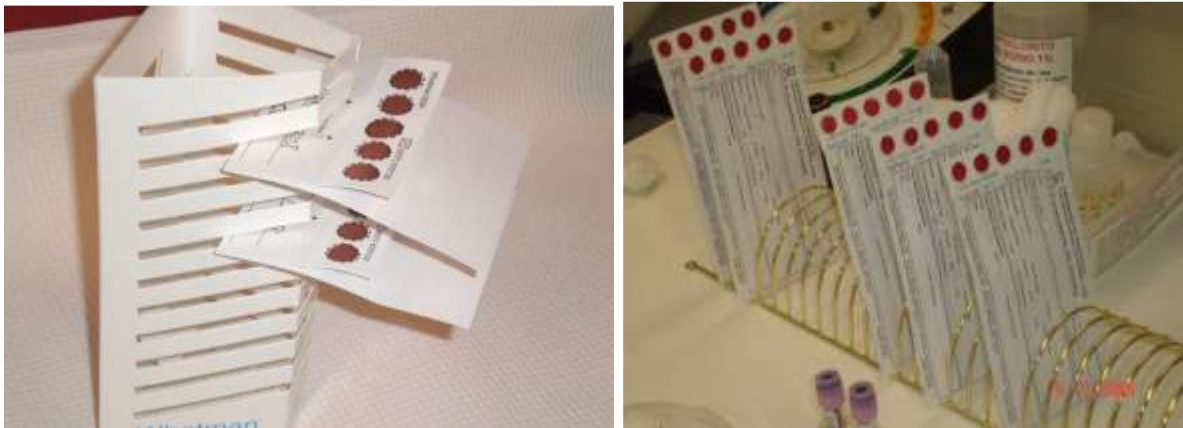


Figure 4. Whatman Protein Saver 903 Card/or any other common drying rack



7.0 Appendices

7.1 Sample collection log

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7.2 Site Specific 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	

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Mbagathi



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

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Kilifi

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

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Kampala

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

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Blantyre

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)

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Civil Hospital

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				

CHAIN BLOOD SPOT COLLECTION SOP (MASTER)



Matlab

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		

CHAIN BLOOD SPOT COLLECTION SOP (MASTER)



Dhaka

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		