CHN 37: CHAIN WHOLE STOOL & RECTAL SWAB PROCESSING SOP

Purpose
The purpose of this SOP is to describe the standard procedures involved in processing and storing of study whole stool samples and rectal swabs.

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 the study laboratory coordinator through your site lab-coordinator.
Main CHAIN laboratory coordinator: Caroline Tigoi (email: ctigoi@kemri-wellcome.org)

Abbreviations/Definitions

SOP Standard Operating Procedure

Materials
1. Fecal collection containers
2. Non-absorbent plastic surface
3. Wooden spatula
4. Patient Sample labels
5. Sample storage vials (2.0 ml)
6. -80 freezer
7. Polycarbonate freezer boxes

Methods
1.0 General considerations
1.1 Samples collected from patients in this study will be for study-specific analyses
1.2 Specimens collected for various tests
1.3 Correct specimen collection bottles and correct request forms must always be used and verified at each collection.
1.4 Ensure all samples should be labelled by Country code, site code, collection time point, (see Site Specific Collection Schedule (appendix 7.2), specimen type (F1, F2 or F3), Patient ID and date of collection. For example: **10-001-A0-F1-XXX-12/10/14**. Add a red sticker to the tubes if caregivers does not consent to international shipping of samples.
1.5 Ensure that tubes marked with a red sticker are stored in a separate freezer box that is clearly labeled (Fecal not for shipment). These samples will be retained at the site as they have no consent for international shipping.
1.6 Keep samples on ice, with ice packs at all times.
1.7 For stool, storage cryovials should be at least 2/3 full (i.e. 1.5ml of stool).
1.8 If the volume of stool is insufficient, the aliquots F1 and F2 have priority.

1.9 Store each aliquot in separate 2 inch polycarbonate (Nalgene 10 x 10 system) freezer box. Samples are destined for different analytic sites for specific analyses and are to be separated at this stage to facilitate an efficient pre-transportation process.

1.10 Each freezer box should be labeled on the top and on the side using printed cryo labels. The label should contain a unique number letter combination (see sample freezer box storage log – Appendix 7.3).

2.0 Whole Stool Processing

2.1 Ensure all fecal collection containers have been correctly labeled, by comparing the sample to the Sample Shipment Log.

2.2 Record time of receiving samples on the Sample Shipment Log. Stool collection pots should contain at least 5ml of stool. Record insufficient stool volume on the CRF.

2.3 Prior to aliquoting the samples, label the empty freezer storage vials with specific barcodes. The first two aliquots will be shipped internationally. These aliquots will be called F1 and F2. The final sample will be retained at the study site, and will be called F3.

2.4 Mix stool using a spatula before dividing it into the different aliquots.

2.5 Stool should be collected in fecal collection containers and separated using the wooden spatula or a plastic wire loop in 3 aliquots of at least 1.5 ml or 2/3 full of a cryovial (named F1, F2 & F3). If volume is insufficient, fill the first two tubes and label accordingly. F1 should be stored in labelled crovial filled with 1.0 ml of freezing mixture and care must be taken not to overfill the tube (See SOP CHN59). Ensure that the freezing mixture is not expired and that it is always stored in a fridge/cool box at 2 to 8^{o}C. Freezing mixture should not be used after one month from the preparation date.

2.6 Patient samples which arrive at the laboratory with a red sticker indicate that these samples must never be shipped, because the patient has not consented to international shipping. If you receive a sample WITH a red sticker, confirm with the study team that this sample is correctly labelled (i.e., the family has not consented to international shipping). All three aliquots should be barcoded, and a red sticker also placed on the cryovials. They should however be stored at the site in a designated clearly labeled cryobox i.e. “Fecal not for shipment”.

2.7 Ideally, stool samples should be stored at -80 degrees Celsius within 30 minutes after arrival at the laboratory. However, blood and rectal swabs can be processed prior to stool samples which may result in stool having a processing time of over 30 minutes. Never discard a stool sample because the processing time has been too long. Please record time of storage in the Sample shipment Log and the CRF.

3.0 Rectal swab processing

3.1 Ensure all rectal swabs have been correctly labeled. By comparing the sample to the Sample shipment log.

3.2 Patient samples which arrive at the laboratory with a red sticker do not have consent for international shipping. Confirm with the study team that this sample is correctly labelled with the red sticker (i.e., the family has not consented to international shipping). All three aliquots, from these patients should be clearly labelled with a red cryo sticker confirming that they should not be shipped.

3.3 Rectal swabs R1 collected using a dry FLOQswab should be cut just below the shaft and

stored in a 2 ml cryotube. It should be stored in -80 degrees Celsius within 30 mins of arrival at the laboratory. Record time of storage in the Sample Shipment Log.

3.4 Rectal swab R2 should be put in 1ml of freezing mixture (See SOP CHN59) and should be stored up right in -80 degrees Celsius within 30 minutes of arrival at the laboratory. If R2 is being used for culture on site, then it will be transported in carry Blair available as a kit together with the swab and it should be cultured within 30 minutes of receipt in the laboratory (See CHN101).

3.5 All the isolates obtained after culture should be stored in – 80^{o}C freezers (3 morphologically different isolates for E. coli and one each for other isolates).Each Isolate should be labelled with Country code, site code, Isolate name (E.coli 1, E.coli 2 or E.coli 3), Patient ID and date of isolation. **10-001-E.coli 1-XXX-12/10/14**.

4.0 Sample log and registration

4.1 At the laboratory the stool sample are divided into 3 aliquots and rectal swabs transferred to 2 ml cryotubes before storage. The Sample shipment Log MUST be filled out. Complete this log immediately after the samples have been placed in the freezer

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

5.0 Document history

Version 1	Author	Approved by	Dated	SOP No:
1.03 **CHAIN Stool sample processing**	Kirk tickellk	Caroline Tigoi	10/11/2016	**CHN37**
1.04 **CHAIN Stool sample processing**	Kirk tickellk	Caroline Tigoi	06/01/2017	**CHN37**

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	1st Jan 2016	DM

7.0 Appendices

7.1 Sample shipment log

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

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

SITE NAME:		**STUDY NAME:**			**SAMPLE COLLECTION POINT:**	
RECIEVING LAB:		**PI NAME:**			**DATE:**	
Subject ID	**Specimen Type***	**Specimen ID (Barcode number)**	**Visit No****	**Date Collected**	**Time collected**	**Comments**

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 (Plasma, Serum or DBS) or Rectal Swab (R1 and R2)

Appendix 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

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 RDT	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 7.3: Sample freezer box storage log

CHAIN Stool 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									