Standard Operating Procedures for the LAKANA trial
SOP Lab-11 Processing of blood, urine and stool samples
Version 1.0. (2022-11-15)

1. Purpose and overview:

This Standard Operating Procedure (SOP[1]) explains how to process and store plasma, PBMCs, urine and stool samples following receipt at the laboratory for the growth sub-study of the LAKANA trial.

2. Applicability to and responsibilities of various staff members

Staff member	Responsibility
Driver/messenger	- Transporting collected samples to the designed laboratory in cooler box
Laboratory technician	- Maintaining enough storage materials in the laboratory - Receiving samples - Processing and storage of specimen using good aseptic technique - Accurately recording the specimens that are processed

3. Required materials

Item	Number	Specification
Disinfectant	1	10% bleach and 70% ethanol 1 % virkon
Centrifuge	1	with Swinging Bucket Rotor and Tube Carriers/Adapters for 13 x 100mm Tube Size and can generating at least 1500 RCF at the tube bottom.
Single-channel Pipettors	As required	100 µl, 1000 µl
Sample wood stick	1/participant	15 cm, sterile
Cryogenic 2 ml tube	10/participant	SARSTEDT CryoPure Tube or similar ones
Specimen lab printed labels	10/participant	None
Box for samples	As required	For the -80 ºC freezer
Biohazard waste containers	1	None
Pasteur pipette	1/blood sample	
15 mL conical tube	2/blood sample	
Trypan blue	As required	
Freezing medium	As required	
Mr. Frosty freezing container	As required	18 samples/container (2 ml tube)
Liquid Nitrogen Freezer and tanks		

[1] Abbreviations: DCF = Data collection form, LAKANA = Large-scale assessment of the key health-promoting activities of two new mass drug administration regimens with azithromycin, PBMC = peripheral blood mononuclear cell, PID = participant identification, SOP = Standard operating procedure

4. Definitions and general instructions

4.1. Definitions

4.1.1. Driver/messenger: Driver and/or a messenger who is responsible for biological sample transportation from the sample collection site to a laboratory.

4.1.2. Laboratory technician: a staff member in the laboratory responsible for LAKANA study for receiving samples, processing and storage of samples.

4.2. General Instructions

4.2.1. The following biological samples will be processed and stored

Note: Box number starting with 6001 indicates for MDA6. Similarly, box number will start with 7001 for MDA7, 8001 for MDA8 and 9001 for visit 9.

Sample	**label**	**Box number**	**Temperature (ºC)**
Plasma	Vial 11	6001 - 6030	-80
Plasma	Vial 12	6031 - 6060	-80
Cells	Vial 13	6061 - 6090	Mr. Frosty, Liquid nitrogen
Cells	Vial 14	6091 - 6120	Mr. Frosty, Liquid nitrogen
Urine	Vial 15	6121 - 6150	-80
Urine	Vial 16	6151 - 6180	-80
Urine	Vial 17	6181 - 6210	-80
Whole stool sample	Vial 18	6211 - 6240	-80
Whole stool sample	Vial 19	6241 - 6270	-80
Whole stool sample	Vial 20	6271 - 6300	-80
Extra cells	Vial 21-	6301 -	Mr. Frosty, Liquid nitrogen

4.2.2. **The aliquots should be aliquoted away from bacterial cultures and the area needs to be wiped down with disinfectant (1% virkon) between samples.**

5. Step-by-step procedures

5.1. Reception and initial processing of the samples at the testing laboratory

5.1.1. At Kita and Bamako lab: During handover of the samples (there should be 1 BD CPT blood tube per participant at 18-25°C box, and 1 urine container and 1 stool container per participant at 2-8°C cooler box) from the driver/messenger, laboratory technician cross-checks that all Data Collection Form (DCF) 13 (Biological sample collection) in tablet or 13c-Biological Sample Collection-growth sub-study (Appendix 1), sample logbook (Appendix 2) and respective samples have matching identifiers. If they match, the laboratory technician will proceed to the next step. If they are not matching, the laboratory technician will contact the nurse who collected the sample and filled DCF13 or 13c to resolve discrepancies. If discrepancies cannot be solved, the sample will be disposed.

Note: Remember to keep the blood tube at 18-25°C until centrifuge and protect tubes from direct light, urine and stool samples in 2-8°C.

5.1.2. Bamako lab: Laboratory technician then fills the laboratory sample reception form (Appendix 3) and follow the SOP for the reception and handling of biological samples (Appendix 4). S/he will record the blood, urine and stool sample in the laboratory logbook - FreezerPro (LAKANA, growth sub-study, participant identification (PID), date and time of receipt at the laboratory). If there is a problem regarding any of the sample reception form questions, the information of each problematic sample will be written in an excel file with the identifiers and the specific problem.

Note: If there is no FreezerPro software or there is connection issue, laboratory technician will record the above information in the logbook file in the computer (e.g. excel) or in the manual logbook which can be later uploaded or input to FreezerPro.

5.2. Blood sample processing

Note: FCS/10% DMSO which is used to resuspend PBMCs prior to freezing must be cooled to 4 °C. This is because DMSO is toxic to cells at room temperature. Place cryovials, Mr. Frosty, and Freezing Media at 4°C to chill before processing.

5.2.1. Remix the blood sample immediately prior to centrifuge by gently inverting the tube 8-10 times. Also, check to see that the tubes are in the proper centrifuge carrier/adapter. Centrifuge blood tube at room temperature (18-25°C) in a horizontal rotor (swing-out head) for 25 minutes at 1500 RCF (Relative Centrifugal Force).

To calculate the correct centrifuge speed for a given RCF, use the following formula:

$$\text{RPM Speed Setting} = \sqrt{\frac{(\text{RCF}) \times (100{,}000)}{(1.12) \times (r)}}$$

Where r (expressed in centimeters) is the radial distance from the centrifuge center post to the tube bottom, when the tube is in the horizontal position and RCF is the desired centrifugal force, 1500 in this case.

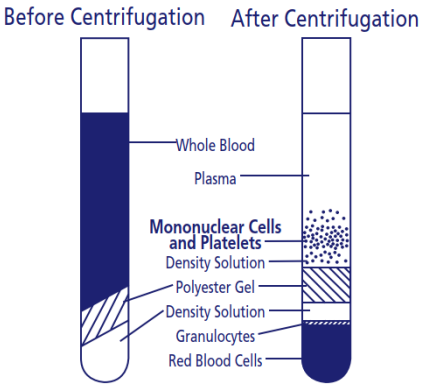
Note: If the red blood cells do not go down in the bottom, increase centrifugal force to maximum 1800 and centrifuge time to 30 minutes.

5.2.2. Print 4 new barcode label stickers with the study acronym (LAKANA-MDA6), PID number, visit time, vial number (vials 11 - 14), sample type and the sample unique ID (from the barcode label sticker on the sample tube). If barcodes are not available to write labels by hand and record in a logbook.

Note: In case the barcode labels are not available, write labels by hand with the information above on the sample tube and record it in FreezerPro or a logbook.

5.2.3. After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer (see Figure below). Affix two new barcode labels on two empty 2 ml tubes (vial 11-12). Aspirate approximately 500 µl plasma twice without disturbing the cell layer and put them in the two 2 ml tubes (vial 11 and 12).

Layering of Formed Elements in the BD Vacutainer® CPT™ Tube



5.2.4. Collect cell layer with a Pasteur pipette and transfer to a 15 mL size conical centrifuge tube with cap labeled with sample ID. Collection of cells immediately following centrifugation will yield best results.

5.2.5. Cell washing, counting and storage:

5.2.5.1. Add PBS to bring volume to 15 ml. Cap tube. Mix cells by inverting tube 5 times.

5.2.5.2. Centrifuge for 15 mins at 300 RCF. Aspirate as much supernatant as possible without disturbing cell pellet.

5.2.5.3. Resuspend cell pellet in PBS to bring the volume to 10 ml. Transfer 20 µl of the resuspended cells and 80 µl of trypan blue to an Eppendorf tube and count.

5.2.5.4. Record and enter the number of viable cells, the number of dead cells which are blue as they have taken up thrypan blue in "CVD-Mali Cell Count Worksheet" electronic form.

5.2.5.5. Repeat the wash. Resuspend cell pellet by pipetting up and down or tapping tube with index finger. Cap tube. Mix cells by inverting tube 5 times. Centrifuge for 10 mins at 300 RCF. Aspirate as much supernatant as possible without disturbing cell pellet.

5.2.5.6. Affix two new barcode labels on two empty 2 ml cyrovials (vial 13-14).

5.2.5.7. Adjust cell concentration to 3-5×10^6 cells/ml with cold FCS/10% DMSO and resuspend the cells by gently pipetting up and down with the Pasteur pipette. Transfer 1 ml of PBMCs into each of the cyrovials. If there are more cells, transfer 1 ml of PBMCs into each extra 2 ml cryovials accordingly. If there are less cell than 3×10^6 cells/ml, freeze any cells that there down in one vial. Gently wipe the outside of the tubes with disinfectant (1% virkon) and place them in a cold Mr. Frosty.

Note: DMSO is toxic to cells at room temperature, therefore it must be added cold. The cells should not be left at room temperature for any longer than is necessary.

5.2.5.8. Immediately transfer Mr. Frosty to the -80 °C freezer and freeze at a minimum of overnight.

5.2.5.9. Once the vials are frozen, put the two vials and extra vial into separate freezer boxes (see 4.2.1.) and transfer to liquid nitrogen freezer for storage.

5.2.6. Fill out the FreezerPro or map boxes record for plasma and cell aliquot samples.

5.3. Urine sample processing

5.3.1. Print 3 new barcode label stickers with the study acronym (LAKANA-MDA6), PID number, vial number (vials 15 - 17), sample type and the sample unique ID (from the barcode label sticker on the sample tube). See details in 5.2.2.

5.3.2. Affix three new barcode label (vial 15-17) on three empty 2 ml tubes. Make 3 urine aliquots. Mix the urine sample and take 1.5 ml urine into each tube. If there is not enough urine for 3 aliquots, fill as many aliquots as possible, and mark in an excel that there was not enough urine, with the sample identifiers.

5.4. Stool sample processing

5.4.1. Print 3 new barcode label stickers with the study acronym (LAKANA-MDA6), PID number, vial number (vials 18 - 20), sample type and the sample

unique ID (from the barcode label sticker on the sample tube). See details in 5.2.2.

5.4.2. Affix three new barcode label (vial 18-20) on three empty 2 ml tubes. Mix the stool and take some stool using the spoon attached to the stool container and put them into each of the above cryovials with altogether 1-1.5 g stool samples in each vial. If stool sample sticks on the spoon, use wood stick to get stool sample into the tube. If there is not enough stool for 3 aliquots, fill as many aliquots as possible, and mark in an excel that there was not enough stool, with the sample identifiers.

5.5. Storage of sample aliquots

5.5.1. Place the aliquots vials into the designed boxes (see 4.2.1) after each sample type aliquot and clean all working surfaces with 10% bleach followed by 70% alcohol.

5.5.2. Each freezer box must have a label on both the box and lid. Freeze boxes in the upright position. Boxes must contain the following information:

1) Trial acronym
2) Box number
3) Specimen type – Vial number and Collection time point
4) Date range of samples in the box

Example:

LAKANA-AMR	LAKANA-AMR
Box 6001	Box 6031
Plasma – vial 11, MDA6	Plasma – vial 12, MDA6
22Aug2022 –	22Aug2022 –

5.5.3. Place the sample boxes in a rack (the same vial number in the same box and same vial boxes in the same rack) and store the racks in a -80^{o}C freezer. Record the location of each vial in the freezer plan.

6. Occupational Safety Issues

6.1. All study team members undertaking this SOP must be trained in good clinical laboratory practice

6.2. All study team members will handle all rectal specimen with care and treat them as potentially infectious material.

7. Quality Assurance / Quality Control

All involved study personnel who will process samples will undergo practical training. Study personnel will not be approved to process samples until a laboratory supervisor has assessed their competency and signed off in the training log.

8. Appendices and other related documents

Document number	Document content
Appendix 1	Data Collection Form (DCF) 13c-Biological Sample Collection-growth sub-study
Appendix 2	Sample logbook
Appendix 3	Laboratory Sample Reception Form
Appendix 4	SOP – Reception of Biological Samples

9. Version history, authors and approvals

Version (date)	Edits to the SOP text (author)
1.0 (2022-11-15)	Drafted by Awa Traore, Dagmar Alber, Jane Juma and Yuemei Fan, approved by PSG.

Appendix 1. Form 13c: Biological Sample Collection-growth sub-study

Section Header	Question Text	Question Responses	Required
Form 13c — Biological Sample Collection	Instructions: Complete this form for targeted age group children.		
	Interviewer ID (filled in automatically)		Yes
	Child ID (scan from child ID sticker)		Yes
A. VISIT INFORMATION	1. Date		Yes
	2. MDA round (Visit number)	6S | 7S | 8S | 9S	Yes
	3. Sample collection place	Village central place/pop-up facility	Yes
	4. Child age group	6-8 mo | 12-14 mo	Yes
B. SAMPLE COLLECTION	5. What samples collected?	Urine | Stool | Venous blood	Yes
	6. Was a stool sample collected?	Yes | No	Yes
	6a. What time the whole stool sample was collected?		Yes
	6b. What date and time did the child pass the stool?		Yes
	6c. Identifier (barcode) of the stool sample		Yes
	7. Was a urine sample collected?	Yes | No	Yes
	7a. What time the urine sample was collected?		Yes
	7b. Identifier (barcode) of the urine sample		Yes
	8. Was a blood sample collected?	Yes | No	Yes
	8a. What time the venous blood sample was taken?		Yes
	8b. Identifier (barcode) of the blood sample		Yes

Appendix 2. Sample logbook

Study name: LAKANA-growth sub-study

Village name: _____

Study nurse (sample collector): _____

Date: _____
Date Month Year

Count Number	Participant ID	MDA number	Sample type	Sample ID (barcode No.)	Collection time	Temp. of cooler box	Name of Driver/messenger	Name of lab recipient
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								

Appendix 3. Laboratory Sample Reception Form

CENTRE POUR LE DEVELOPPEMENT DES VACCINS-MALI (CVD-MALI)

Département de Microbiologie et de Biologie Moléculaire

LABORATORY SAMPLE RECEPTION FORM

Participant ID:…… **Site:**……

Sample collection

Date:……/……/……..(DD/MM/YYY)

Time:……/…… (24H00)

Sample reception in the laboratory

Date:……/……/…… (DD/MM/YYY)

Time:……/…… (24H00)

Sample accepting/ rejection criteria

Q1. Is sample properly labeled?	Yes	◯	No	◯
Q2. Is sample container tightly shut?	Yes	◯	No	◯
Q3. Is the temperature adequate (2-8°C)?	Yes	◯	No	◯
Q4. Is the sample collection time to delivery in the lab adequate (within 72 hours)?	Yes	◯	No	◯
Q5. Does the information on the CRF match the information on the sample?	Yes	◯	No	◯
Q6. Is the sample acceptable for processing?	Yes	◯	No	◯

Laboratory Technician: …… **Date:**……/……/…… **(DD/MM/YYY)**

Appendix 4. SOP – Reception of Biological Samples

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STANDARD OPERATING PROCEDURE

Reception of Biological samples

1. Objective

This standard operating procedure provides instructions for the reception and handling of biological study samples for laboratory analysis and storage.

2. Responsibilities

- The laboratory director or his representative is responsible for ensuring that the protocol is carried out as indicated.
- The laboratory director or his representative is responsible for ensuring that personnel are trained in the execution of this protocol.
- The laboratory technician is responsible for carrying out the procedure precisely and on time.

3. Documents

Interim guidelines from the Centers for Disease Control and Prevention (CDC) for the collection, handling and analysis of clinical samples

4. Materials

- Tube holder / rack
- 9 x 9 or 10 x 10 sample storage boxes
- Refrigerator (2-8°C)
- -80°C Freezer
- Personal Protective Equipment (PPE)
- Labels for sample aliquots
- 1ml pipette tips
- Plastic Pasteur pipettes
- Micropipette 1ml
- Vortex
- 1.5ml Sarstedt tubes
- Class II Biological Safety Cabinet (BSL-2)
- 10% Bleach / Virkon
- 70% Ethanol
- Scissors

5. Biosafety

- All biological samples must be considered hazardous and must be treated and processed according to appropriate biosafety guidelines.
- Appropriate PPE must always be worn before handling and manipulating samples.
- Work benches, equipment and materials must be cleaned with 10% bleach followed by 70% Ethanol before and after work.
- All materials that come into contact with the samples must be disposed off in accordance with biosafety guidelines.
- Biohazard waste should be disposed off appropriately following biosafety guidelines

6. Procedure

- Wash your hands with soap and running water and dry them with tissue or sanitize hands with alcohol-based hand sanitizers.
- Wear appropriate personal protective equipment (PPE) including a lab coat, goggles, face shield, nasal masks, gloves and protective footwear as necessary before touching / handling the samples.
- Use 10% bleach followed by 70% ethanol to clean work benches and the biological safety cabinet
- Prepare 10% bleach or virkon in a disinfect jar and place it in the biosafety cabinet
- Clean a tube holder/ rack with 10% bleach and 70% ethanol
- Place the tube holder/ rack in the biosafety cabinet
- Check the date and time of sample collection on the sample accompanying form. **Note:** this should be in compliance with study protocol.
- Carefully open the cooler and check the temperature at which the samples were transported
- Record the temperature on the sample receipt form.
- Remove and place the packed sample inside a BSL-2 safety cabinet.
- Check the sample against the accepting/ rejection criteria below

i. that the temperature is adequate (2-8°C)
ii. that the sample container is tightly shut.
iii. that the specimen is correctly labeled and that the information on the sample container corresponds with the information on the accompanying lab request form.
iv. that the sample volume is adequate as per study requirements
v. that the sample collection and transportation is in compliance with the study protocol

- If all the above conditions are met, proceed with the reception of the samples. Otherwise, keep the sample at 2-8 °C in a refrigerator and inform the sample collection and submitting teams to resolve issues. If issues cannot be resolved, reject the sample and inform the clinical study team.
- In a BSL-2 cabinet, proceed to aliquot samples in tubes (1.5ml or 2ml) according to study protocol. If aliquoting is not required, process/ test the samples as required by the study protocol, proceed to label and store the whole sample under the required conditions.
- Print barcode sample labels and stick them to the different aliqouts as per

protocol.

- Use the appropriate sample inventory software e.g. FreezerPro, Global Trace etc. to scan in the samples into their proper positions in the sample box and proceed to store the sample under the required conditions.
- Take the samples to the designated freezer in the cold room and place them in their corresponding positions in the sample box as in the storage inventory.
- Store samples for long-term storage at -80°C
- Complete a sample reception form if required by the study
- Discard biological waste in the appropriate biohazard containers
- Clean work benches and the biological safety cabinet with 10% bleach followed by 70% ethanol
- Carefully remove and dispose off PPE. Disinfect reusable PPE with 10% bleach followed by 70% ethanol and store them appropriately.
- Wash your hands thoroughly with soap and running water.
- Dry hands with tissue and dispose them in the trash bags.
- Disinfect hands with hand sanitizing gel