Standard Operating Procedures for the LAKANA trial

**SOP Lab-09 Processing of whole stool specimen**

Version 1.0. (2022-03-15)

# Purpose and overview:

This Standard Operating Procedure (SOP[[1]](#footnote-1)) explains how to process and store whole stool specimens following receipt at the laboratory for the mechanistic 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 and printing new cryovial barcode label * 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 |
| Cryogenic 1.8 mL screw cap tube (Blue cap) | 1/participant | SARSTEDT CryoPure Tube |
| Cryogenic 1.8 mL screw cap tube (Yellow cap) | 1/participant | SARSTEDT CryoPure Tube |
| Cryogenic 1.8 mL screw cap tube (Green cap) | 1/participant | SARSTEDT CryoPure Tube |
| Cryogenic 1.8 mL screw cap tube (Violet cap) | 1/participant | SARSTEDT CryoPure Tube |
| Sample wood stick | 1/participant | 15 cm |
| Specimen barcode labels | 4/participant | None |
| Biohazard waste containers | 1 | None |

# 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

| Sample | **label** | **Box number** | **Temperature (oC)** |
| --- | --- | --- | --- |
| Whole stool sample | Vial 7  (blue cap) | 4001 - 4040 | -80 |
| Whole stool sample | Vial 8  (yellow cap) | 4041 - 4080 | -80 |
| Whole stool sample | Vial 9  (green cap) | 4081 - 4120 | -80 |
| Whole stool sample | Vial 10  (violet cap) | 4121 - 4160 | -80 |

# 5. Step-by-step procedures

## 5.1. Reception and initial processing of the whole stool specimen at the testing laboratory

### 5.1.1. At Kita and Bamako lab: During handover of the stool specimen (there should be 1 stool container per participant) from the driver/messenger, laboratory technician cross-checks that all Data Collection Form (DCF) 13 (Biological sample collection) in tablet or 13b-Biological Sample Collection-mechanistic sub-study (Appendix 1), sample logbook (Appendix 2) and respective stool specimen 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 13b to resolve discrepancies. If discrepancies can not be solved, the sample will be disposed.

### **Note:** Remember to put the samples in 2-8 oC until the stool samples are in Bamako.

### 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 stool specimen in the laboratory logbook - FreezerPro (LAKANA, mechanistic sub-study, participant identification (PID), date and time of receipt at the laboratory and minimum and maximum temperatures since being placed in the cooler box). 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.

### Print 4 new barcode label stickers with the study acronym (LAKANA-MDA4), PID number, visit time, vial number (vials 7 - 10), 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.

### Prepare 4 cryovials (each with different color cap – blue, yellow, green and violet).

### Affix one new barcode label sticker (vial 7) on the empty blue cap 1.8mL vial.

### Affix one new barcode label sticker (vial 8) on the empty yellow cap 1.8mL vial.

### Affix one new barcode label sticker (vial 9) on the empty green cap 1.8mL vial.

### Affix one new barcode label sticker (vial 10) on the empty violet cap 1.8mL vial.

### Make 4 stool sample aliquots. 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. There is a line at 1.0 ml and at 1.8 ml, fill the aliquot between those two lines. If stool sample sticks on the spoon, use wood stick to get stool sample into the tube. If there is not enough stool for 4 aliquots, fill as many aliquots as possible, and mark in an excel that there was not enough stool, with the sample identifiers.

* + 1. Place the 4 vials (vials 7 - 10) to the designed boxes (see 4.2.1).
    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) Assays sample will be used for

5) Date range of samples in the box

Example:

LAKANA-AMR LAKANA-AMR

Box 4001 Box 4041

Stool - vial 7, MDA4 Stool - vial 8, MDA4

14Feb2022 – 28Feb2022 Feb2022 – 28Feb2022

LAKANA-AMR LAKANA-AMR

Box 4081 Box 4121

Stool - vial 9, MDA4 Stool – Vial 10, MDA4

14Feb2022 – 28Feb2022 14Feb2022 – 28Feb2022

* + 1. Clean all working surfaces with 10% bleach followed by 70% alcohol.

### Place the sample boxes in a rack (the same vial number and color cap in the same box and same vial boxes in the same rack) and store the racks in a -80oC freezer. Record the location of each vial in the freezer plan.

# Occupational Safety Issues

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

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

# Quality Assurance / Quality Control

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

# Appendices and other related documents

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

# Version history, authors and approvals

| Version (date) | Edits to the SOP text (author) |
| --- | --- |
| 0.1 (2022-01-30) | Authored by Yuemei Fan, Dagmar Alber, Rikhard Ihamuotila, Jane Juma and Awa Traore. |
| (2022-03-15) | Authored by Yuemei Fan, Dagmar Alber, Rikhard Ihamuotila, Jane Juma and Awa Traore. |

**Appendix 1. Form 13b: Biological Sample Collection-mechanistic sub-study**

|  |  |  |  |
| --- | --- | --- | --- |
| **Section Header** | **Question Text** | **Question Responses** | **Required** |
| Form 13b — Biological Sample Collection-mechanistic sub-study | Instructions: Complete this form for targeted age group children. | | |
|  | Interviewer ID (study nurse ID) |  | Yes |
|  | Child ID (child ID sticker) |  | Yes |
| A. VISIT INFORMATION | 1. Date |  | Yes |
|  | 2. MDA round (Visit number) | 4S-00 / 4S-14 | Yes |
|  | 3. Sample collection place | Village central place/pop-up facility |  |
|  | 4. Child age group | 4-11 mo | Yes |
| B. SAMPLE COLLECTION | 5. What samples collected? | Heel prick blood CRP | Heel prick blood Hb | Heel prick blood, blood spot | Stool | Yes |
|  | 6. Was a heel prick blood sample collected? | Yes | No | Yes |
|  | 6a. Record the result of CRP | CRP = | Yes |
|  | 6b. Record the result of Hb | Hb = | Yes |
|  | 7. How many blood spots were stored on filter paper card? | 0 | 1 | 2 | Yes |
|  | 7a. Identifier (barcode) of the filter paper card? |  | Yes |
|  | 8. Was a stool sample collected? | Yes | No | Yes |
|  | 8a. What time the whole stool sample was collected? |  | Yes |
|  | 8b. What date and time did the child pass the stool? |  | Yes |
|  | 8c. Identifier (barcode) of the stool sample |  | Yes |

**Appendix 2. Sample logbook**

**Study name: LAKANA-Mechanistic 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**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| amali-hyphen   |  | | --- | |  | |  |  |  |  |  |  |  |  |
|  |  | **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**

|  |  |  |
| --- | --- | --- |
| **CENTRE POUR LE DEVELOPPEMENT DES VACCINS-MALI (CVD-MALI)** | **Code** |  |
| **Version** | 2 |
| **From** | MAY 2020 |
| **Next Review** | MAY 2021 |
| **Pages** | 2 |
| **Authorizer’s**  **signature** |  |

**STANDARD OPERATING PROCEDURE**

**Reception of Biological samples**

# Objective

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

# 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.

# Documents

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

# 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

# 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

# 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

1. that the temperature is adequate (2-8°C)
2. that the sample container is tightly shut.
3. that the specimen is correctly labeled and that the information on the sample container corresponds with the information on the accompanying lab request form.
4. that the sample volume is adequate as per study requirements
5. 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

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, PID = participant identification, SOP = Standard operating procedure [↑](#footnote-ref-1)