Standard Operating Procedures for the LAKANA trial
SOP Lab-03 Processing of rectal swab specimen
Version 2.0 (2021-04-20)

1. Purpose and overview:

This Standard Operating Procedure (SOP[1]) explains how to process and store rectal swab specimens following receipt at the laboratory for the antimicrobial resistance (AMR) 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 labels - Storage of rectal specimens using good aseptic techniques - Accurately recording the specimens that are processed

3. Required materials

Item	Number	Specification
Disinfectant	1	10% bleach and 70% ethanol
Vortex	1	None
Permanent marker	1	None
Specimen barcode labels	3/participant	None
Biohazard waste containers	1	None

[1] Abbreviations: AMR = antimicrobial resistance, DCF = Data collection form, DESS = DMSO/EDTA/saturated sodium chloride, 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

4. Definitions and general instructions

4.1. Definitions

4.1.1. Driver/messenger: Driver and/or a messenger who are 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 receiving samples, storage of LAKANA study samples.

4.2. General Instructions

4.2.1. The following biological samples will be stored at each time point

Sample	**label**	**Temperature (ºC)**
Swab in Cary Blair media	Vial 1	-80 or 2-8 if cultured within 48h
Swab in DESS media	Vial 2	-80
Dry rectal swab	Vial 3	-80

5. Step-by-step procedures

5.1. Reception and initial processing of the rectal specimen at the testing laboratory

5.1.1. During handover of the rectal specimen (there should be 3 per participant) from the driver/messenger, laboratory technician cross-checks that all Data Collection Form (DCF) 13 (Biological sample collection) in tablet or 13a-AMR (Appendix 1), sample logbook (Appendix 2) and respective rectal 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 13a-AMR to resolve discrepancies. If discrepancies can not be solved, the sample will be disposed.

5.1.2. Laboratory technician then fill the laboratory sample reception form (Appendix 3) and follows the SOP for the reception and handling of biological samples (Appendix 4). S/he will record the rectal specimen in the laboratory logbook - FreezerPro (project name, AMR, visit number, sample type, participant identification (PID) number, date and time of receipt at the laboratory and minimum and maximum temperatures since being placed in the cooler box).

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.

5.1.3. Print 3 new barcode label stickers with the study acronym (LAKANA-AMR), PID number, visit time, vial number (vial 1-3), sample type and the sample unique ID (from the barcode label sticker on the sample tube).

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 a logbook.

5.1.4. Affix a new barcode label sticker (vial 1) on the Cary Blair media with rectal swab vial and the DESS media with rectal swab vial (vial 2).

5.1.5. Affix the last new barcode label sticker (vial 3) on the received 2mL vial with a dry swab in it.

5.1.6. Clean all working surfaces with 10% bleach followed by 70% ethanol.

5.1.7. Vortex the sample tubes received with Cary Blair media (vial 1) containing the rectal specimen for 30 seconds to disperse the bacteria on to the Cary Blair media.

5.1.8. Place all 3 vials (vials 1-3) in a -80^{o}C freezer, if batch *E. coli* culture and AMR testing will be carried out at a later stage. Record the location of each vial in the freezer plan or a logbook.

5.1.9. If *E. coli* culture from the rectal sample will be carried out within 48 hours, vial 1 with Cary Blair media can be temporarily placed at 2-8°C until ready to culture (not exceeding 48 hours from sample collection). All other vials (vials 2 and 3) should be frozen at -80^{o}C.

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 rectal 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.

8. Appendices and other related documents

Document number	Document content
Appendix 1	Data Collection Form (DCF) 13a-AMR
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)
2.0 (2021-04-20)	Changed vial number (no aliquot for Cary Blair vial) and deleted required material and corresponding text for making aliquot. Approved by LAKANA PSG on April 20, 2021.

Version (date)	**Edits to the SOP text (author)**
1.0 (2021-03-09)	Authored by Dagmar Alber, Elaine Cloutman-Green and Yuemei Fan. Approved by LAKANA PSG on March 09, 2021.

Appendix 1. Data Collection Form (DCF) 13a-AMR

Section Header	Question Text	Question Responses	Required
Form 13a — Biological Sample Collection-AMR	Instructions: Complete this form for targeted age group children (4-14 mo and 49-59 mo children).		
	Interviewer ID (study nurse ID)		Yes
	Child ID (child ID sticker)		Yes
A. VISIT INFORMATION	1. Date		Yes
	2. MDA round (Visit number)		Yes
	3. Sample collection place	Village central place/pop-up facility	
	4. Child age group	4-14 mo | 49-59 mo	Yes
B. SAMPLE COLLECTION	5. What samples collected?	Rectal swab | Nasopharyngeal swab	Yes
	6. How many rectal swabs were collected?	0 | 1 | 2 | 3	Yes
	6a. What time the rectal swabs were collected?		Yes
	6b. Identifier (barcode) of the first rectal swab in Cary-Blair medium tube		Yes
	6c. Identifier (barcode) of the second rectal swab in DESS medium tube		Yes
	6d. Identifier (barcode) of the third dry rectal swab		Yes
	7. How many nasopharyngeal swabs were collected in STGG media?	0 | 1	Yes
	7a. What time the nasopharyngeal swab was collected?		Yes
	7b. Identifier (barcode) of the nasopharyngeal swab		Yes

Appendix 2. Sample logbook

Study name: LAKANA-AMR

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

	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

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