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

1. Purpose and overview:

This Standard Operating Procedure (SOP[1]) explains how to process and store nasopharyngeal swab (NPS) 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 label - Processing and storage of NPS 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
Vortex	1	None
Pipette	1	P1000
Pipette Tips	1/participant	1mL
Cryogenic tubes or 2mL screw cap tube with o-ring	1/participant	None
Permanent marker	1	None
Specimen barcode labels	2/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.

[1] Abbreviations: AMR = antimicrobial resistance, DCF = Data collection form, LAKANA = Large-scale assessment of the key health-promoting activities of two new mass drug administration regimens with azithromycin, NPS = Nasopharyngeal swab, PID = participant identification, SOP = Standard operating procedure

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 at each time point

Sample	**label**	**Temperature (°C)**
Swab in STGG media	Vial 4	-80 or 2-8 if cultured within 48h
	Vial 5	-80

5. Step-by-step procedures

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

5.1.1. During handover of the NPS specimen (there should be 1 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 NPS 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 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 NPS specimen in the laboratory logbook - FreezerPro (LAKANA, AMR, participant identification (PID), 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 2 new barcode label stickers with the study acronym (LAKANA-AMR), PID number, visit time, vial number (vial 4 and vial 5), 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 a logbook.

5.1.4. Affix one new barcode label sticker (vial 5) on the received 2mL vial with STGG media and NPS swab in it.

5.1.5. Affix the other new barcode label sticker (vial 4) on an empty 2 mL vial.

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

5.1.7. Vortex the sample tube received with STGG vial containing the NPS specimen (vial 5) for 30 seconds.

5.1.8. Using a P1000 pipette and under aseptic biosafety conditions, transfer 400 µL of the vortexed STGG sample (vial 5) into the labeled empty cryovial (vial 4).

5.1.9. Discard the used pipette tip into 10% bleach.

5.1.10. Place the 2 vials (vial 4 and vial 5) in a -80°C freezer, if batch *Streptococcus pneumoniae* culture and AMR testing will be carried out at a later stage. Record the location of each vial in the freezer plan.

5.1.11. If *Streptococcus pneumoniae* culture from the NPS sample will be carried out within 48 hours, vial 4 can be temporarily placed at 2-8°C until ready to culture (not exceeding 48 hours from sample collection). Vial 5 should be frozen at -80°C and will serve as a back-up.

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 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.

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 from 5 and 6 to vial 4 and 5. Approved by LAKANA PSG on April 20, 2021.
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)	**de**	
	rsion	
	)m	AY 2020
	xt Review	AY 2021
	ges	
	thorizer's nature	

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