Standard Operating Procedures for the LAKANA trial
SOP Proc-05 Collection of rectal swab samples
Version 1.0 (2021-03-23)

1. Purpose and overview:

This Standard Operating Procedure (SOP[1]) provides step-by-step instructions on how to collect rectal swab specimen from children at 4-14 months and 49-59 months in the household participating in the antimicrobial resistance (AMR) sub-study of the LAKANA trial. This SOP refers to data collection forms (DCF) 13 – Biological sample collection. The purpose of this SOP is to ensure an optimal and standardized approach to the collection of rectal swab specimen.

Following the described procedure will maximize the validity and reliability of antimicrobial resistance data obtained in the LAKANA trial.

2. Applicability to and responsibilities of various staff members

Staff member	Responsibility
Study nurse	- Coordinating the setting up of the pop-up facility in village - Facilitating timely collection of rectal specimens from children enrolled in the AMR sub-study - Collecting and ensuring rectal specimens from enrolled children are correctly labelled - Completing DCF 13 – biological sample collection forms in tablet computer or in paper form 13a-AMR (Appendix 1) - Ensuring the specimen log is complete, up to date and transported to the laboratory
Driver/messenger	- Transporting the nurse and sample collection material between CSCom and village - Assisting the nurse in setting up of the pop-up facility - Transporting collected samples to the designed laboratory in cooler box
Relais	- Accompanying participating children and their caregivers to pop-up facility - Assisting the nurse in handling children at pop-up facility

[1] Abbreviations: AMR = Antimicrobial resistance, COVID-19 = coronavirus disease 2019, 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, DCF = Data collection form, MDA = mass drug administration, NPS = nasopharyngeal swab, PID = participant identification, PPE = personal protective equipment, SOP = Standard operating procedure

3. Required materials

Item	Number	Specification
Sample bags	1 bag/participant	Each sample bag includes two smaller zip bags. Use the "Rectal" one. It includes 3 flocked swabs, one 2mL tube with 1mL Cary Blair media, one 2mL tube with 1mL DESS, and one empty 2mL tube, each tube with barcode label sticker on it.
Extra bag of rectal swabs	1 bag/village	Extra bag of swabs – one bag of swabs 520CS01 (20pcs) for rectal specimen collection
Disposable gloves	2 pairs/participant	None
Baby wipe	1 wipe/participant	None
Biohazard waste bag	1	None
Ice packs	*Number required to meet the daily target for sample temporary storage*	For maintaining cool box at 2-8°C
Cooler box	1	For transporting rectal specimens (such as Coleman 9 Quart Excursion Cooler or DOMETIC CFX3 35)
Min/max-temperature monitors	1/cooler box	For determining whether temperature excursion occurred and for how long (e.g. as MyM Instruments Tecnico Product Number HTC-2 or Fisher Scientific™ Traceable Thermometer 14-648-26)
Surgical mask/ N-95 mask	1	None
Goggles or face shield	1	None
Paper towel	1-2/participant	None
Disinfectant	500 – 1000 mL	10% bleach and 70% alcohol

4. Definitions and general instructions

4.1. Definitions

4.1.1. Study nurse: a LAKANA staff member responsible for AMR and mechanistic sub-study sample collection, sample collection form, sample log and arranging transport to the laboratory.

4.1.2. Eligible children: a child aged 4 – 14 months or 49 – 59 months at the time of study drug provision whose primary caregiver provides a permission for the procedure.

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

4.1.4. Relais Communautaire: a volunteer chosen by the community who serves as a bridge between professional health staff and the villagers.

4.2. General instructions

4.2.1. During the COVID-19 epidemic, physical distancing will be enforced: a distance of at least 1 meter will be maintained between any two individuals (exception will apply when the swab is collected).

4.2.2. The data collection team members will always wear a mask when in a village.

4.2.3. Rectal specimen will be collected from selected study participants who are participating in an AMR sub-study. The specimen will be taken at the enrollment BEFORE the study medication is administered (visit 1, MDA1), 12 months (visit 5, MDA5), 24 months (visit 9) and 36 months (visit 11), at village pop-up facility.

4.2.4. Prior to the rectal specimen collection, the study nurse will complete the following preparatory steps:

4.2.4.1. When the study participants and their caregiver arrive at village pop-up facility, the study nurse will check information and fill in DCF13 form (Biological sample collection) in the tablet according to the instruction of the corresponding study visit SOP. If scanning the child ID information is not working, fill in the DCF13a-AMR paper form (Appendix 1).

Note: Study nurse will ONLY fill sample collection form DCF13a-AMR paper version WHEN the electronic DCF13 in the TABLET is NOT working.

4.2.4.2. Explain the procedure clearly to the child's caregiver and the child. The procedure is quick and harmless but sometimes causes some discomfort. This procedure requires the help of the caregiver to obtain a good sample. It is important to describe the procedure to the parent/caregiver so that they can best assist with keeping the child still during the procedure. Three rectal swabs will be collected.

4.2.4.3. Prepare the workspace to ensure that the rectal specimen will not become contaminated. Wash or sanitize your hands. Put on a pair of gloves. Clean the work surface with disinfectant and paper towel. All necessary material (sample bag, gloves and so on) should be kept over the cleaned workspace where they are easily accessible and there is no risk of contamination.

4.2.4.4. Take one sample bag from the cooler box and get the rectal bag and put the NP bag back to cooler box. Open the rectal bag and place the vials in a rack in the following order: Cary Blair vial, DESS vial and empty

vial. This is the order the samples will be taken. Slightly loosen the lid making sure the content (Clary Blair media or DESS) does not spill. Prepare sample logbook which includes study name, village name, date, participant identification (PID) number, time point (MDA number or visit number), sample type, sample ID (e.g. number with barcode label), collection date and time, cooler box temperature, name of sample carrying person (driver/messenger) and lab receiving person (Appendix 2).

Note: Study nurse will ALWAYS fill the logbook for each sample collected.

4.2.4.5. Emphasize the importance of remaining still during specimen collection to minimize discomfort.

4.2.5. After rectal specimen collection, the study nurse will make sure that all samples are appropriately labelled and coordinate with driver/messenger to deliver ideally within 4 hours to the laboratory for processing and storage. Samples should be collected within 48 hours provided they have been stored at 2-8°C.

4.2.6. Appropriate specimen collection devices, containers, and transport media must be used to ensure optimal recovery of microorganisms and storage.

5. Step-by-step procedures

5.1. Sample collection

5.1.1. Give the parent or guardian a baby wipe to wipe the child's bottom prior to sample collection.

5.1.2. Ask the parent or caregiver to hold the child so that s/he is lying on her/his side, with hips and knees flexed.

5.1.3. Remove the first swab from its packaging. Take care that the tip does not touch anything. If it does come into contact with anything discard and use a new swab.

5.1.4. Insert the tip of the swab into the child's anus only as far as needed to contact faecal material (approximately 3 cm deep) and gently rotate the swab back and forth at180 degrees for 15 seconds and pull the swab out of the anus. The tip should be a brownish color when removed.

5.1.5. Place the swab containing the sample in the vial containing the Cary Blair (CB) media. Press the swab to the bottom of the vial and twist and break of the plastic handle sheath by placing the sheath against the side of the tube. Tighten the screw cap of the tube to prevent any spillage.

5.1.6. Put the excess swab stick and other disposable material in the waste bag.

5.1.7. Repeat 5.1.3-5.1.4 for the second swab and place it in the vial containing the DESS media. Snap of the plastic handle sheath and tighten the tube to prevent any spillage.

5.1.8. Put the excess swab stick and other disposable material in the waste bag.

5.1.9. Repeat 5.1.3-5.1.4 for the third swab and place in the empty 2mL screw cap tube. Close the lid of the vial.

5.1.10. Make sure all the vials are correctly labelled (see 4.2.4.4) before placing the three vials in a sample bag and then in a cooler box with ice packs or well-conditioned accumulators and a thermometer (+2 to +8°C).

5.1.11. After collecting the rectal swab specimen, study nurse completes the DCF 13 – biological sample collection in the tablet or paper form DCF 13a-AMR (Appendix 1). Study nurse also completes sample logbook which includes study name, village name, date, participant identification (PID) number, time point (MDA number or visit number), sample type, sample ID (e.g. number with barcode label), collection date and time, cooler box temperature, name of sample carrying person (driver/messenger) and lab receiving person (Appendix 2).

Note: Study nurse will ONLY fill sample collection form DCF13a-AMR paper version WHEN the electronic DCF13 in the TABLET is NOT working. Study nurse will ALWAYS fill the logbook for each sample collected.

If there is no barcode label on the vial, study nurse will write the child's PID number, sample type, date manually on the vial.

5.1.12. A driver/messenger will transport the cooler box with the rectal swab specimen vials to the testing laboratory as soon as possible (If this is not possible, preferably not exceeding 4 hours from time of specimen collection). If this is not possible, samples should be transported within 48 hours provided they have been stored at 2-8°C.

6. Occupational Safety Issues

6.1. During the COVID-19 epidemic, procedures for safe and proper work will be used to reduce the risk of exposure to a hazard and prevent transmission between the study team and the study participants. Special considerations due to COVID-19 are presented *SOP-Safety 01 Hygiene and PPE*.

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

6.3. Wear disposable gloves and change gloves after each study participant.

6.4. Wash or sanitize hands before putting on and after removing gloves.

6.5. All study team members will handle all specimens with care and treat them as potentially infectious material.

6.6. Dispose all contaminated waste (gloves, paper, swab handles, etc.) into biohazard waste bags for incineration or disposal.

7. Quality Assurance / Quality Control

All involved study personnel who will collect specimens will undergo practical training for rectal sample collection. Study nurse will not be approved to collect rectal specimen until a supervising clinician 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

9. Version history, authors and approvals

Version (date)	Edits to the SOP text (author)
1.0 (2021-03-23)	Authored by Dagmar Alber, Elaine Cloutman-Green and Yuemei Fan. Approved by LAKANA PSG.

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								