

Standard Operating Procedures for the LAKANA trial
SOP Proc-06 Collection of nasopharyngeal swab samples
 Version 1.0 (2021-03-23)

1. Purpose and overview:

This Standard Operating Procedures (SOP¹) provides step-by-step instructions on how to collect nasopharyngeal swab (NPS) specimens from children and household contacts 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 NPS 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	<ul style="list-style-type: none"> - Coordinating the setting up of the pop-up facility in village - Facilitating timely collection of NPS specimens from children enrolled in the AMR sub-study - Collecting and ensuring NPS specimens from enrolled children are correctly labelled - Completing DCF 13 – biological sample collection form 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	<ul style="list-style-type: none"> - Transporting the nurse and sample collection material between CCom and village - Assisting the nurse in setting up of the pop-up facility - Transporting collected samples to the designed laboratory in cooler box
Relais	<ul style="list-style-type: none"> - Guiding participating infants and 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, 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 bag	1 bag/participant	Each sample bag includes two smaller zip bags. Use the “NP” one. It includes 1 flocked nasopharyngeal swab, one 2mL tube with 1mL STGG media with barcode label sticker on it.
Extra bag of nasopharyngeal swabs	1 bag/village	Extra bag of swabs – one bag of swabs 503CS01 (20pcs) for NPS specimen collection
Scissors	1 pair/participant	For cutting swabs shaft after taking sample
70% ethanol or 70% ethanol wipes	500 ml or 10-20 wipes/village	To clean scissors if they are contaminated
Biohazard waste bag	1	None
Cooler box	1	For transporting STGG media containing tube and NP specimens (such as Coleman 9 Quart Excursion Cooler or DOMETIC CFX3 35)
Ice pack	<i>Number required to meet the daily target for sample temporary storage</i>	For maintaining cool box temperature at 2-8°C
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)
Disposable gloves	1 pair/participant	None
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% ethanol

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 wear a mask at all times when in a village.
- 4.2.3. NPS specimen will be collected from selected study participants who are participating in an AMR sub-study. The specimens 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 NPS 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 if applicable. The procedure is quick and harmless but sometimes causes some irritation that may lead the child to withdraw from the swab or shed some tears.
 - 4.2.4.3. Prepare the workspace to ensure that the NPS specimen will not become contaminated. Wash or sanitize your hands. Put on the personal protective equipment (PPE), the mask shield and gloves. Clean the work surface with disinfectant and paper towel. All necessary material (sample bag, gloves, scissor wiped with 70% ethanol 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 the NP bag from the cooler box, the rectal bag from the same sample bag is already used for sample collection from the same participant. Place the vial in a rack. Slightly loosen the lid making sure the content (STGG media) 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. Occasionally the NPS specimen procedure may cause a small amount of bleeding and this can make the NPS appear blood-tinged.
- 4.2.5.** After the NPS specimen collection, the study nurse will assess the participant's respiratory status and rule out trauma after the procedure. Address any clinical issues as indicated.
- 4.2.6.** After the NPS 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 storage and further processing. Samples should be collected within 48 hours provided they have been stored at 2-8°C.
- 4.2.7.** 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 (please see also video demonstrations, appendix 3-4)

5.1.1. Children on parent/caregiver's lap.

The parent / caregiver should use one arm to hold the child's arms and the other arm should be placed on the child's forehead. (Fig 1)



Fig 1: holding child on lap during NPS collection

- 5.1.2.** Parents may sit chest to chest for smaller children (If the parent and the child are sitting chest to chest, the child's head should be positioned over one of the parent's shoulders, and the parent should have one hand on the child's head and the other arm across their back (Fig 2). Study personnel may hold the child at the request of the parent/caregiver.



Fig 2: holding child over the shoulder during NPS collection

- 5.1.3.** The distance from the nose to the ear gives an estimate of the distance the swab should be inserted. (Fig 3). The swab should be inserted half that distance



Fig 3: Measurement of insertion of swab. The swab should be inserted half that distance.

- 5.1.4.** Tilt the patient's head back at a 70 degree angle. (Fig 4)

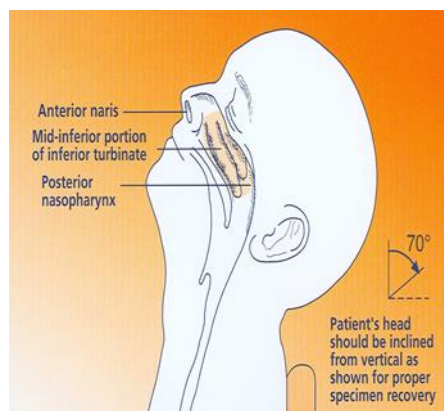


Fig 4: Tilt head by 70°

- 5.1.5.** Remove the flocked swab from its protective package.
- 5.1.6.** Be careful not to touch anything with the tip of the swab.
- 5.1.7.** Ensure the vial cap faces upwards during taking swab (not touching the surface).

- 5.1.8.** Insert the swab with the nylon tip into one nostril horizontally (not upwards) and continue along the floor of the nasal passage for several centimeters until reaching the nasopharynx (resistance will be met). Note that if the swab is

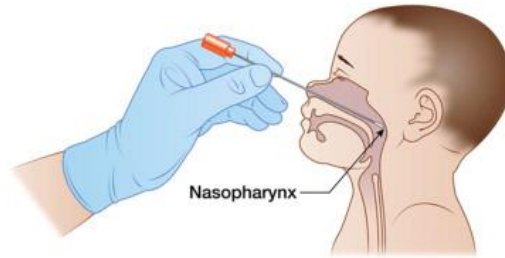


Fig 5: Insertion of swab

not perpendicular to the frontal plane of the face, it is likely not in the inferior turbinate (Fig 5).

- 5.1.9.** Do not force the swab. If obstruction is encountered before reaching the nasopharynx, remove the swab and try the other side.
- 5.1.10.** Once you reach the nasopharynx, rotate the swab gently 180° 2-3 times to make sure adequate specimen is obtained.
- 5.1.11.** Leave the swab in place for 3-5 seconds to ensure maximum absorbance of secretions.
- 5.1.12.** Remove swab and immediately dip the swab tip vertically into the labelled STGG media vial making sure not to spill the media. Press the swab to the bottom of the vial, use a pair of clean scissors (previously wiped with 70% ethanol) to cut off the shaft so the swab fits into the tube containing the STGG media. Close the lid of the vial. (Fig 6).

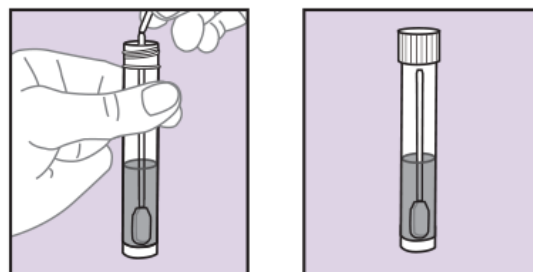


Fig 6: placing swab in vial and cutting off the shaft

- 5.1.13.** Put the excess swab stick and other disposable material in the waste bag.
- 5.1.14.** Tighten the lid on the vial to prevent spillage and place the vial in test tube rack and then in a thermos or a cool box with ice packs or well-conditioned accumulators and a thermometer (+2 to +8°C).
- 5.1.15.** After collecting the NPS specimen, study nurse completes DCF 13 – Biological sample collection form 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.

- 5.1.16.** A driver/messenger will transport the cooler box with the NPS specimen vial 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 specimen 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 specimen will undergo practical training for NPS collection. Study nurse will not be approved to collect NPS 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
Appendix 3	Video demonstrations of NPS collection procedure. https://youtu.be/ar2Grm_t8X8 https://www.youtube.com/watch?v=DVJNWefmHjE
Appendix 4	Review the "Swab Clinical Specimen" video on the CDC website: http://www.cdc.gov/pertussis/clinical/diagnostic-testing/specimen-collection.html

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

