

Standard Operating Procedures for the LAKANA trial

## SOP Proc-04 Preparation of sampling bags for collection of rectal and nasopharyngeal swab samples

Version 2.0 (2021-04-20)

### 1. Purpose and overview:

This Standard Operating Procedure (SOP<sup>1</sup>) describes how to assemble a sample pack for the collection of rectal and nasopharyngeal swab samples in the antimicrobial resistance (AMR) sub-study of the LAKANA trial.

### 2. Applicability to and responsibilities of various staff members

Staff member	Responsibility
Laboratory technician/scientist	<ul style="list-style-type: none"><li>- Maintaining enough materials and reagents</li><li>- Preparing reagents</li><li>- Preparing the sampling packs</li><li>- Keeping track of expiry dates</li><li>- Sending the sampling packs to sample collection team</li></ul>

### 3. Required materials

Item	Number	Specification
Flocked swab	4 swabs/sample pack	For rectal specimen collection 3 swabs (Copan swab with 30mm breakpoint – 520CS01) and for nasopharyngeal specimen collection one NPS (Copan swab with 100mm breakpoint – 503CS01)
Extra bag of Flocked swabs	2 bags/village	Extra bags of swabs – one bag of swabs 520CS01 (20pcs) for rectal specimen collection, one bag of swabs 503CS01 (20pcs) for nasopharyngeal specimen collection
2mL screw cap tube with o-ring	4 tubes/sample pack	2mL screw cap tubes such as Sarstedt (72.694.406)
STGG Media	1 mL/sample pack	STGG can be made locally, Standardized, see appendix 1

<sup>1</sup> Abbreviations: AMR = Antimicrobial resistance, CB = Cary Blair, CSCoM = Centre de Santé Communautaire, 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, NP = Nasopharyngeal, SOP = Standard operating procedure, STGG = a medium containing skim milk, tryptone, glucose and glycerin

Item	Number	Specification
Cary Blair Media	1 mL/sample pack	Cary Blair Media can be made locally, see appendix 2
Disposable gloves	Change as needed	None
DESS medium	1 mL/ sample pack	DMSO/EDTA/saturated sodium chloride, see appendix 3
Micropipette	1	For dispensing 1mL media
Tips	3 at least, 1 for STGG, 1 for Cary Blair media and 1 for DESS. Change as needed	1000µL
Specimen label	4/ sample pack	None
Sample bags	3/sample pack	None
Disinfectant	500 – 1000 mL	10% bleach and 70% ethanol

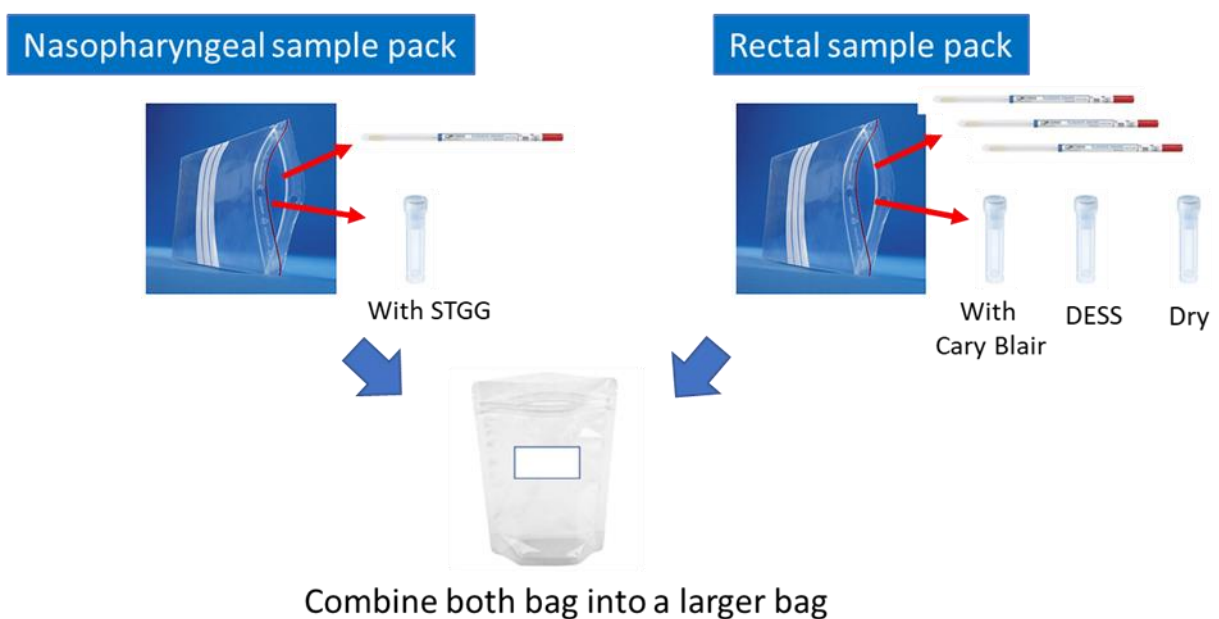
**4. Definitions and general instructions**

**4.1. Definitions**

**4.1.1.** Laboratory technician/Scientist: a LAKANA staff member responsible for AMR and mechanistic sub-study sample collection, sample log and arranging transport to the laboratory.

**4.2. General Instructions**

Diagram on how to assemble collection pack



## **5. Step-by-step procedures**

### **5.1. Preparation of media and aliquoting**

- 5.1.1.** Prepare STGG as outlined in Appendix 1.
- 5.1.2.** Prepare Cary Blair medium as outlined in Appendix 2.
- 5.1.3.** Prepare DESS medium as outlined in Appendix 3.
- 5.1.4.** If media has been made in advance and stored in a refrigerator, check expiration date and make sure media is not contaminated (clear, not cloudy).
- 5.1.5.** Using aseptic techniques aliquot 1mL of STGG media into a 2mL sterile screw cap tube and close lid tightly. Label tube with STGG.
- 5.1.6.** Using aseptic techniques aliquot 1mL of Cary Blair media into a 2mL sterile screw cap tube and close lid tightly. Label tube with CB.
- 5.1.7.** Using aseptic techniques aliquot 1mL of DESS media into a 2mL sterile screw cap tube and close lid tightly. Label tube with DESS.
- 5.1.8.** If media has been aliquoted into tubes in advance and stored in a refrigerator, check expiration date and make sure media is not contaminated (clear, not cloudy, salt precipitation may be present which should not influence the performance of DESS).
- 5.1.9.** Repeat as necessary.

### **5.2. Assembling nasopharyngeal pack**

- 5.2.1.** Label 1 zip bag with 'NP'. Place a blank sticker on the bag.
- 5.2.2.** Label 1 sterile screw cap tube with 'STGG' and affix a pre-printed unique barcode label sticker on it. The 'STGG' will be written on both cap and top part of the tube to make sure it can be seen clearly with a barcode label on it. The field team will scan the barcode to the tablet when the sample is collected.
- 5.2.3.** Place the labelled STGG tube and one individual packed nasopharyngeal swab (Copan swab - 503CS01) in the labelled 'NP' bag and close.

### **5.3. Assembling rectal pack**

- 5.3.1.** Label 1 zip bag with 'Rectal'. Place a blank sticker on the bag.
- 5.3.2.** Label 1 sterile screw cap tube with 'CB' and affix a pre-printed unique barcode label sticker on it. The 'CB' will be written on both cap and top part of the tube to make sure it can be seen clearly with a barcode label on it. The field team will scan the barcode to the tablet when the sample is collected.

- 5.3.3. Label 1 sterile screw cap tube with 'DESS' and affix a pre-printed unique barcode label sticker on it. The 'DESS' will be written on both cap and top part of the tube to make sure it can be seen clearly with a barcode label on it. The field team will scan the barcode to the tablet when the sample is collected.
- 5.3.4. Affix a pre-printed unique barcode label sticker on another empty sterile 2mL screw cap tube.
- 5.3.5. Place the labelled CB tube, the DESS tube, empty sterile tube and 3 swabs with 30 mm breakpoint (Copan swab – 520CS01), in the labelled 'Rectal' bag and close.
- 5.4. **Assembling of sample collection bag**
  - 5.4.1. Affix a blank sticker on a large zip bag.
  - 5.4.2. Place the 'NP' bag and 'Rectal' bag in the large zip bag, making sure all bags have the material mentioned above and close.
  - 5.4.3. The collection bag is now ready. They should be kept at 2-8°C and to send to the CSComs and from there to villages in cooler box at 2-8°C.
- 5.5. **Assembling of extra bags of flocked swabs**
  - 5.5.1. Label 1 zip bag with 'extra NP swabs'.
  - 5.5.2. Place 20 individual packed nasopharyngeal swab (Copan swab - 503CS01) in the labelled 'extra NP swabs' bag and close.
  - 5.5.3. Label 1 zip bag with 'extra rectal swabs'.
  - 5.5.4. Place 20 swabs with 30 mm breakpoint (Copan swab – 520CS01), in the labelled 'extra rectal swabs' bag and close.

**Note:** Before the barcode label could be printed, the lab personnel will use pre-handwritten label together with study nurse to label the collected sample vial.

- In the future, the lab personnel will start to pre-handwritten label the sample vials when they prepare the sample bags in the lab. Please see the following format, the text in black written by the lab personnel and **red part by nurse in the field**. The lab personnel will leave space on the label for study nurse to fill.

**LAKANA-AMR**

**MDA1, medium** (CB, DESS, dry or STGG)

**Sample type** (Rectal swab or NPS) – **vial number** (1, 2, 3 or 5)

**Child ID**

**Child age** (age group in months)

**Date of sample collection**

- If the sample bags are already dispatched from the lab to the field without pre-handwritten information, the study nurse needs to pre-handwritten label the following information on the sample vials using thin-point pen  
**LAKANA-AMR, MDA1 (or short version LA1)**  
**Child ID (MUST be clear and recognizable)**  
**Child age (age group in months)**  
**Date of sample collection**
- If the sample vials collected from the same child are in the bigger bag, the detailed information should be also marked on the bigger bag, so the lab personnel can recognize the samples easily.
- The sample logbook filled by study nurse should be transported together with collected samples to the Kita lab. The lab personnel must check the sample vials and logbook to see if they are match and take a photo of logbook page he/she received for cross-checking the information marked on the sample vial.

## 6. Occupational Safety Issues

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

## 7. Quality Assurance / Quality Control

All involved study personnel who will prepare the sample bags will undergo practical training. Study personnel will not be approved to assemble bags until a supervisor has assessed their competency and signed off in the training log.

## 8. Appendices and other related documents

Document number	Document content
Appendix 1	Preparation of STGG
Appendix 2	Preparation of Cary Blair medium
Appendix 3	Preparation of DESS

## 9. Version history, authors and approvals

Version (date)	Edits to the SOP text (author)
2.0 (2021-04-20)	Added “Note” instruction for handwritten sample vial label information by lab personnel and study nurse when barcode labels are not available. 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 Preparation of STGG**

### For 100 ml STGG media

#### Ingredients:

1. skim milk - 2 gm
2. trypticase soya broth – 3 gm
3. glucose – 0.5 gm
4. glycerol – 10 ml
5. distilled water – 90 ml

#### Procedure

All ingredients will be mixed together in an autoclavable container and autoclaved at temperature of 116°C, under 15 lbs pressure for 15 minutes. STGG is allowed to cool to room temperature. Date the medium and give it a batch number. Record the expiry date (six months from day of preparation). The medium is now ready for aseptically aliquoting into 2 ml screw cap vials. Media or tubes prepared in advance should be stored in a refrigerator at 2°-8°C. Prior to aliquoting, vortex the media for 20 seconds.

If possible, the entire 3000 mL could be made with same media batch and make several packs.

Test the media 1 week before the batch is used for sterility by plating the entire volume of one vial from each batch number onto Trypticase soy agar with 5% sheep blood and incubating the plate at 37°C for 48 h in a CO<sub>2</sub> incubator. If the growth of any organism is observed, discard the batch.

## **Appendix 2. Preparation of Cary Blair medium**

### Ingredients:

Thermo Scientific Cary-Blair Transport Medium (Dehydrated)

Distilled water

Prepare medium according to the manufacturer's instructions. Date the medium and give it a batch number. Record the expiry date (1 year from the date of preparation). The medium is now ready for aseptically aliquoting into 2ml screw cap vials. Media or tubes prepared in advance should be stored in a refrigerator at 2°-8°C.

If possible, the entire 3000 mL could be made with same media batch and make several packs.

Test the media 1 week before the batch is used for sterility by plating the entire volume of one vial from each batch number onto Trypticase soy agar with 5% sheep blood and incubating the plate at 37°C for 48 h in a CO<sub>2</sub> incubator. If the growth of any organism is observed, discard the batch.

### **Appendix 3. Preparation of DMSO/EDTA/saturated sodium chloride (DESS)**

To make 0.5M EDTA if not available:

Disodium EDTA FW 374.24	372.24g
5M NaOH	To pH EDTA to 8.0
Deionized water	Bring final volume to 2L

1. Measure out 372.24g of disodium EDTA, add 500mL deionized water to the EDTA, and enough 5M NaOH to pH the solution to 8.0 (this can be as much as 500mL in some cases). Please note EDTA will not dissolve if the pH is below pH7.0. Therefore add enough 5M NaOH to dissolve the EDTA. This may take several hours. Once fully dissolved, adjust the pH to 8.0 using a pH meter or pH paper.

Make sure to use disodium EDTA salt otherwise more NaOH is needed to pH the EDTA.

2. Bring the final volume to 2L with deionized water.

For 2L DESS media

Ingredients:

1. 0.5M disodium EDTA – 1L
2. Dimethyl Sulfoxide (DMSO) – 400mL
3. Deionized water (sterile) – 600 mL
4. NaCl – Enough to saturate the solution ~300g

Procedure

Measure out and mix the first three chemicals.

Add enough NaCl to saturate the solution (roughly 300-400g, this may vary depending on several factors including ambient temperature, etc so is best to add 300 grams and if everything dissolves then add more salt until it seems like it just isn't dissolving anymore) and dissolve. This may take several minutes to hours, so be patient.

Make sure to store the solution in an airtight container in the dark (wrap bottle in aluminum foil). Appearance of crystals is normal and should not influence performance. However,



make sure that the solution does not become cloudy. If this happens discard and make up a fresh one.