Standard Operating Procedures for the LAKANA trial
SOP Proc-09: Collecting blood, urine, and stool samples at the pop-up facility
Version 1.0 (2022-11-15)

1. Purpose and overview:

This SOP[1] gives step-by-step instructions on how to collect blood, urine, and stool samples in the sub-study visit 06, 07, 08 for the growth sub-study (samples to also serve for the mechanistic sub-study outcome measures) of LAKANA in selected sub-study/AMR villages near the city of Kita. As a reminder, the mechanistic sub-study aims at identifying the mechanisms behind mortality reduction with MDA and other effects of MDA on child growth and biomedical markers.

2. Applicability to and responsibilities of various staff members

Staff member	Responsibility
Nurse	- Handles and prepares the sample collection material. - Collects biological samples from 6-8 mo. and 12-14 mo. children. - Records sample-related data in study app. - Ensures samples are correctly labelled, transported to and deposited in Kita/Bamako lab. - Treats eligible 6-8 mo. infants after sample collection. - Disposes of study waste.
Laboratory technician/scientist	- Maintains enough materials - Prepares the sampling material together with pre-printing barcode labels - Keeps track of expiry dates - Sends the sampling material and barcode labels to the sample collection team

3. Required materials

Item	Number	Specification
Tent	1	A pop-up medical tent to be set up in the village.
Table	1	Rent from village.
Chairs	3	Rent from village.
Clinical waste bin	1	To be kept at the pop-up facility.
Nurse's Tablet computer	2	The following questionnaires will be loaded: DCF02b (treatment), DCF13c

[1] Abbreviations: SOP = standard operating procedure, DCF = data collection form, AMR = antimicrobial resistance, CSCom = Centre de Santé Communautaire, LAKANA = Large-scale assessment of the key health-promoting activities of two new mass drug administration regimens with azithromycin, MDA = mass drug administration.

Item	Number	Specification
		(sample collection), DCF06a (preliminary information on child death)
Pre-printed barcode sticker	3	One for each sample
DCF13c, hard copy Appendix 1	*As required*	The paper version DCF13c needs to be filled by study nurse for sample collection if Tablet computer or electronical DCF13 are not functional.
Sample logbook, hard copy Appendix 2	*As required*	The study nurse ALWAYS fill the sample logbook for each sample collected.
Disposable gloves	2 pairs/participant *Number required to meet the daily target*	For cleaning the skin.
Cleaning wipes	*Number required to meet the daily target*	For cleaning the skin around the perineum and genitals.
BP Vacutainer CPT	*Number required to meet the daily target*	For keeping the blood samples.
Needle	*Number required to meet the daily target*	For piercing the vein for sample collection.
Tourniquet	*1*	For holding back venous return.
Sharp disposal box	*1*	For disposing sharp needle waste
Adhesive bandage	*Number required to meet the daily target*	To cover tightly the needle wound in order to stop the bleeding and prevent infections of the needle prick area.
Alcoholic wipes	*Number required to meet the daily target*	For cleaning the skin before blood sampling.
Stool container	*Number required to meet the daily target*	For collecting the stool.
Urine collection bag	*Number required to meet the daily target*	For collecting the urine.
Urine container	*Number required to meet the daily target*	Container to hold the sample.

Item	Number	Specification
Cold box 2-8 °C degrees	*Number required to meet the daily target*	To store stool and urine samples
Cold box 18-25 °C degrees	*Number required to meet the daily target*	To store blood samples before centrifugal.
Upright holder for blood sample tubes	*Number required to meet the daily target*	To keep the blood sample tubes upright for transportation.
Sterile gauzes	*Number required to meet the daily target*	To apply pressure on the venipuncture site after sampling.
Diapers	*Number required to meet the daily target*	To hold the urine collection bag.
Disinfectant, 70% ethanol	*As required*	To disinfect the surface.

4. Definitions and general instructions

4.1 Definitions

4.1.1. Study nurse: a LAKANA staff member responsible for sub-study sample collection and recording corresponding data in study nurse app.

4.1.2. Laboratory technician/Scientist: a LAKANA staff member responsible for sub-study sample collection material preparation, receiving and processing samples in the laboratory.

4.1.3. Eligible child for the growth sub-study: a child aged 6-8 mo. and 12-14 mo. living in a household that consented to participate in the LAKANA trial growth sub-study.

4.1.4. Caregiver: a person responsible for looking after a child. The caregiver is responsible for providing permission for study drug administration to eligible infants.

4.1.5. Pop-up facility: refers to a temporary clinical facility (a village central place or a tent equipped with appropriate sample collection material) that the LAKANA team will set up in a village for sample collection and anthropometrics measurement purposes.

4.2. General instructions

4.2.1. Blood, urine, and stool samples will be collected at the second day of the MDA6 (7 and 8) visit at the pop-up facility.

5. Step-by-step procedures

5.1. Pre-village activities:

5.1.1. The lab technician will prepare the sample collection material and send them to the field team. He/She will keep track of expiry dates for sample collection materials.

5.2. Preparations at the pop-up facility:

5.2.1. Clean the table in the pop-up facility.

5.2.2. Clean your hands and use disposable gloves.

5.2.3. Place all sample collection material, personal protective equipment, tablet and logbook, and cleaning equipment so that they are easy to reach when needed.

5.2.4. When a participant arrives to the pop-up facility, identify the infant, fill the registration form and scan the ID sticker. Fill the sample logbook and sample collection form to the extent possible. If the scanning of the child ID barcode does not work, information will be filled in the paper version of DCF13c (Appendix 1).

5.3. Collecting blood samples:

5.3.1. Exercise caution when sampling blood due to the risk of bloodborne diseases. Keep surfaces clean, wash hands, and change gloves between each sampling.

5.3.2. Explain the purpose and process of the procedure and explain for what purposes the blood sample is taken and that there is no harm to the child because of it. Ask if they have any questions.

5.3.3. Backflow must be prevented, because if tube content enters the child's bloodstream it can cause severe adverse effects. To prevent backflow bear in mind the following: keep the child's arm in a downward position during sampling, always hold the tube stopper / diaphragm uppermost when sampling, release the tourniquet when the blood starts flowing into the tube, and ensure that the tube contents do not touch the end of the needle or the stopper.

5.3.4. Write the child's name, ID, and date on the blood collection tube. Affix one pre-printed barcode label (start from 6xxxxx for MDA6, 7xxxxx for MDA7, 8xxxxx for MDA8 and 9xxxxx for visit 9) on the tube. Put the ready sterile needle and sample collection tube on the table so that they are easy to grab. Apply then the tourniquet. Clean the puncture site well with an alcoholic

wipe. Do not touch the puncture site after cleaning. Keep the arm downwards and ask for help if the child is moving. Always keep the tube stopper upwards to prevent backflow.

5.3.5. Open the needle shield and perform the venipuncture. Push the tube stopper through the back end of the needle while holding the needle still inside the vein (otherwise the vein might get punctured). When blood starts flowing, immediately remove the tourniquet. Remember to keep the needle immobilized so that the vein is not punctured. Do not let the tube content touch the stopper / diaphragm or back end of the needle. Draw 4 mL of blood.

5.3.6. When an adequate amount of blood is drawn, remove the tube from the needle. If the outer part of the stopper gets contaminated with blood, the tube must be handled as hazardous material. Remove the needle from the vein and apply pressure with clean sterile gauze and use a bandage. **Important: Invert the blood sample tube 8-10 times** to mix anticoagulant additive with blood**.** Do NOT SHAKE. Vigorous mixing can cause hemolysis. Put the needle in the sharp waste bin.

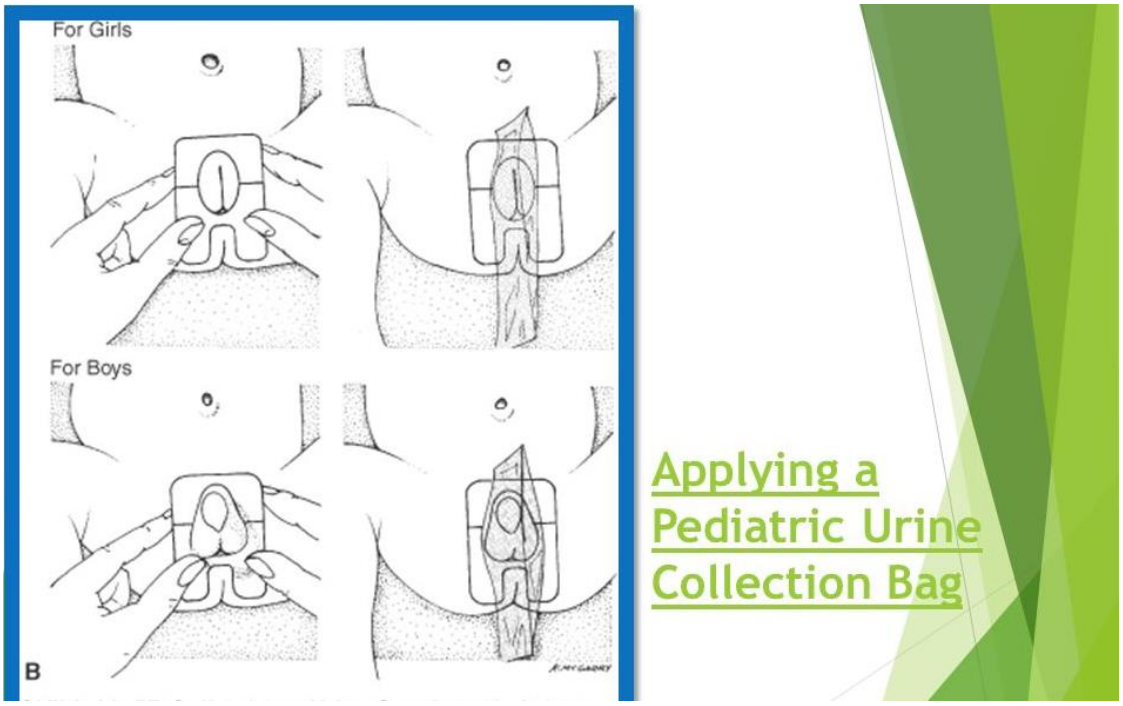
5.3.7. The blood sample tubes must be stored in a 18-25 °C degrees box in an upright position in the holders. Clean area and change gloves if there is any blood contamination. Note: The collected blood sample tubes must be transported to Bamako in maximum 48 hours at 18-25 °C, so the lab can centrifuge and process it.

5.4. Collecting the urine sample:

5.4.1. Explain the purpose and process of the procedure and explain for what purposes the urine sample is taken and that there is no harm to the child because of it.

5.4.2. Clean the areas of the perineum (the area between the genitals and the anus) and genitals and the area between the inner thighs thoroughly with the sterile cleaning wipe soaked in clean warm water. Clean also the urethra. Make sure that there are no patches that were not cleaned.

5.4.3. Attach the urine collection bag starting from the perineum (glue it on the perineum). Continue then to attach it around the scrotum and penis for boys and slightly over (towards the abdomen) the urethra for girls. See picture below and instruction .



5.4.4. Make sure the seal is tight without a leaking point and that it does not pinch the skin. Fold the bag under the child's buttocks and put on a diaper.

5.4.5. Affix one pre-printed barcode label on urine container. Write the child's name, ID, and date on the urine container.

5.4.6. Check every 15 minutes for urine. If there is no urine in 2 hours, the bag must be changed. When there is urine, the bag must be removed as soon as possible to prevent contamination. Peel off the sticking part starting from above and move downwards. Keep the bag hanging downwards. If there is any contamination or stool in the urine, a new sampling should be started from the beginning.

5.4.7. Move the urine in to the urine container with the child name, ID, and date marked on it. Dispose of waste, wash hands, and change gloves. Store the urine sample in 2-8 °C degrees and transport later to Bamako lab for processing and storage.

5.5. Stool sample collection:

5.5.1. Ask the caregiver for the stool sample that was collected at home using the stool container they receive one day earlier. Put one pre-printed barcode label on the stool container, check the child ID and name on the stool container are correct, and scan the barcode. Fill the form in the tablet for stool sample collection. If scanning is not working, enter the barcode number manually in the sample collection form. Put the stool sample in the cooler box for transport later to Bamako lab for processing and storage.

5.5.2. If the caregiver has not collected a stool sample, ask if they can stay at the pop-up facility until a stool sample can be taken.

5.5.3. Store the samples in 2-8 °C degrees.

Urine sample collection instructions were adapted from the Helsinki University Hospital pediatric urine sample collection instructions:

https://huslab.fi/preanalytiikan_kasikirja/virtsanaytteenotto/pussivirtsanaytteenotto.pdf

6. Occupational Safety Issues

6.1. The study nurse will wear disposable gloves when handling a child. S/he will wash or sanitize hands before putting on and after removing gloves. S/he will start by collecting the urine, blood and stool samples and change gloves if the gloves are very bloody before taking the stool sample. If stool sample is taken first for some reason, then hands will be sanitized, and gloves changed before taking blood sample. S/he will change gloves after each study participant.

6.2. All study team members will handle all specimen with care and treat them as potentially infectious material. Appropriate specimen collection devices, containers, and transport media will be used to ensure optimal storage.

6.3. The study nurse will dispose of all contaminated waste (gloves, papers, tubes etc.) into biohazard waste bags for incineration or disposal. All sharp lancets will be disposed in a biohazard sharp waste bin.

6.4. During the COVID-19 pandemic, 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 in *SOP-Safety 01*.

6.5. Blood samples can potentially have dangerous infectious agents such as HIV and hepatitis. Therefore, the correct sampling and handling of blood samples will be checked by the field supervisor for each nurse. Before sampling of the next child, hands will be cleaned, and new gloves will be used. If surfaces or equipment have a risk of contamination, they will be cleaned well with alcohol or be discarded.

7. Quality Assurance / Quality Control

The study nurse who will collect specimen will undergo practical training for blood sample collection. Study nurse will not be approved to collect the specimen until a supervising clinician has assessed their competency and signed off in the training log.

8. Appendices and other related documents

Document number (Version)	Document content
Appendix 1	Data Collection Form (DCF) 13c
Appendix 2	Sample logbook
Appendix 3	*Urine sample collection instructions were adapted from the Helsinki University Hospital pediatric urine sample collection instructions:* https://huslab.fi/preanalytiikan_kasikirja/virtsanaytteenotto/pussivirtsanaytteenotto.pdf

9. Version history, authors and approvals

Version (date)	Edits to the SOP text (author)
1.0 (2022-11-15)	Rikhard Ihamuotila, in consultation with Yuemei Fan, Laura Adubra, Awa Traore, Jane Juma and Fadima Haidara. Approved by PSG.

Appendix 1. LAKANA trial

Form 13c: Biological Sample Collection

Version 0.1, June 27, 2022

Section Header	Question Text	Question Responses	Required
Form 13c — Biological Sample Collection	Instructions: Complete this form for targeted age group children.		
	Interviewer ID (filled in automatically)		Yes
	Child ID (scan from child ID sticker)		Yes
A. VISIT INFORMATION	1. Date		Yes
	2. MDA round (Visit number)	6S | 7S | 8S | 9S	Yes
	3. Sample collection place	Village central place/pop-up facility | CSCom clinic	Yes
	4. Child age group	6-8 mo | 12-14 mo	Yes
B. SAMPLE COLLECTION	5. What samples collected?	Urine | Stool | Venous blood	Yes
	6. Was a stool sample collected?	Yes | No	Yes
	6a. What time the whole stool sample was collected?		Yes
	6b. What date and time did the child pass the stool?		Yes
	6c. Identifier (barcode) of the stool sample		Yes
	7. Was a urine sample collected?	Yes | No	Yes
	7a. What time the urine sample was collected?		Yes
	7b. Identifier (barcode) of the urine sample		Yes
	8. Was a blood sample collected?	Yes | No	Yes
	8a. What time the venous blood sample was taken?		Yes
	8b. Identifier (barcode) of the blood sample		Yes

Appendix 2: Sample logbook

Study name: LAKANA-mechanistic sub-study MDA S4

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

Appendix 3.

Urine sample collection instructions were adapted from the Helsinki University Hospital pediatric urine sample collection instructions:

https://huslab.fi/preanalytiikan_kasikirja/virtsanaytteenotto/pussivirtsanaytteenotto.pdf