Standard Operating Procedures for the LAKANA trial
SOP Proc-08: Measuring CRP and Hb, and collection of DBS and stool samples
Version No. 1.0. (2022-03-15)

1. Purpose and overview:

This SOP[1] gives step-by-step instructions on how to measure CRP (C-reactive protein) and hemoglobin, and how to collect DBS (dry blood spot) and stool samples in the sub-study visit 04 (MDA#4S[2]) for the mechanistic sub-study outcome measures of LAKANA in selected sub-study/AMR villages near the city of Kita. The mechanistic sub-study aims at identifying the mechanisms behind mortality reduction with MDA.

This SOP refers to Data Collection Form (DCF) 13b (Appendix 1).

2. Applicability to and responsibilities of various staff members

Staff member	Responsibility
Nurse	- Handles and prepares the measurement equipment. - Collects biological samples from 4-11 mo. infants. - Ensures samples are correctly labelled, transported to and deposited in Kita/Bamako lab. - Disposes of study waste. - Charges the Quick Read go instrument and one extra battery - Measures the controls of CRP and Hb before each village visit. - Refers the infant to the CSCom if clinical thresholds are crossed.
Laboratory technician/scientist	- Maintains enough materials - Prepares the sampling packs with pre-printing barcode labels (stool container, DBS pack, CRP+Hb kits, CRP and Hb controls) - Keeps track of expiry dates - Sends the sampling packs to the sample collection team - Tests QuikRead go® instrument (CRP+Hb measurement) to make sure each instrument works well before sending them to field

[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.
[2] MDA #4S indicates MDA round 4 for villages included in the Sub-study.

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, DCF13b, DCF06a.
DCF13b, hard copy Appendix 1	*As required*	The paper version DCF13b 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 before and after the blood prick tests (CRP+Hb and dry blood spot on filter paper)
Alcohol wipes	2 wipe/participant *Number required to meet the daily target*	For cleaning the skin before and after the blood prick tests (CRP+Hb and dry blood spot on filter paper)
Adhesive bandage/plaster	*1/participant*	Adhesive bandage 19x72 mm
QuikRead go® instrument	1	For measuring CRP+Hb
Extra battery	1	For QuikRead go® instrument
QuikRead go® wrCRP+Hb reagent kit box	*Number required to meet the daily target*	For measuring CRP and Hb. 1 kit includes 50 tests with capillaries (10 µl) and plungers. Storage of unopened kit at 2-25°C until the expiry date of the kit. Unopened cuvettes after opening the foil pouch: storage at 2-8°C for 6 months, storage at 18-25°C for 3 months.
QuikRead go® wrCRP control	1	Unopened vials at 2-8°C until the expiry date. Opened vial at 2-8°C up to two months.
QuikRead go® Hb control	1	Unopened vials at 2-8°C until the expiry date. Opened vial at 2-8°C up to one months.

Item	Number	Specification
Dry blood spot card	*Number required to meet the daily target*	For collecting dry blood spot and later malaria PCR analyses. PerkinElmer PKI RUO Spot Saver Card
Cooler box	2	Electric cooler box (12v).
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)
Paper towel	1 towel/participant *Number required to meet the daily target*	For cleaning surfaces
Biohazard waste bag	2/village/day	For sample collection and cleaning waste
Disinfectant	2 bottles, 500 ml-1000 ml	10% bleach and 70% ethanol
Surgical mask/ N-95 mask	10 / day	6 for nurse, 2 for data collector, and 2 for relais
Goggles or face shield	1/nurse	For nurse in close contact with patients
Refrigerator	1	To store and maintain transport media at appropriate temperature (2-8°C) and prepare/store ice packs to be used for maintaining cooler boxes.
Lancets for heel prick blood collection	1/participant	For making the skin incision for blood collection. Blood lancets, VITREX® STERIHEEL® Baby II, 2.00x3.00mm
Dry Rak	*Number required to meet the daily target*	Whatman 903 DRY RAK with Velcro, safely and properly air-drying multiple collected DBS specimen cards in a suspended horizontal position.
Plastic zip lock bag	*1 / DBS card*	To pack the DBS card for transport.
Indicating desiccant packet	*1 / sealable bag*	QIAGEN WB100003, 1 g, to keep the DBS card dry in the bag during transport/storage. Desiccant change from blue to pink to indicate absorption of moisture.
Printed barcode label	1 printed label/ stool container (126 labels/ sheet)	For collected whole stool sample, the nurse will affix the label to the contained when received in pop-up facility and fill the participant information and scan the

Item	Number	Specification
	Number required to meet the daily target	barcode (or manually enter if scanning is not working) to tablet
Extra bag of DBS cards	1 bag/village	Extra bag of DBS cards – one bag of DBS cards (20pcs) for DBS specimen collection
Extra bag of desiccant	1 bag/village	Extra bag of desiccants – one bag of desiccants (20pcs) for DBS specimen collection
Instructions to health clinics hard copy Appendix 4	*Number required to meet the daily target*	Instructions for referrals, with the explanation to the referral and clinical guidance recommendations

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. The study nurse must be the same person in all activities.

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

4.1.3. Eligible child for the mechanistic sub-study: a child aged 4-11 mo. living in a household that consented to participate in the LAKANA trial. Consent for participation in the Sub-study will be requested from the head of each household.

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 purposes.

4.2. General instructions

4.2.3. CRP and hemoglobin will be measured, and stool and DBS will be collected in the AMR sub-study villages during MDA4. The samples and measurements will be obtained from the same infants just prior to the study drug administration and again after 2 weeks.

5. Step-by-step procedures

5.1. Pre-village activities:

5.1.1. The lab technician will prepare the sampling packs (pre-printed barcode labels, stool container, DBS, CRP+Hb kits, CRP and Hb controls) and send them to the field team. He/She will keep track of expiry dates for sample collection materials. He/She will test the QuikRead go® instrument before sending it to the field.

5.1.2. The nurse will test the QuikRead go® instrument with CRP and Hb controls before each village visit (not every day). The control measurement is similar to the actual measurement (see below) but uses a control sample instead of real blood. Behind this link is a video on how to perform the control measurement: https://www.youtube.com/watch?v=y4k58rSNfoU

5.1.3. The instrument will be plugged in the charger before the day of the village visit to make sure it is charged for the village activities. One extra battery will be fully charged in advance.

5.2. Preparations at the pop-up facility:

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

5.2.2. Clean your hands and use disposable gloves.

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

5.2.4. Start the QuikRead go® wrCRP+Hb instrument and check that it is functional. If there are any problems with the QuikRead go® wrCRP+Hb instrument, contact the field supervisor or consult the manual booklet.

5.2.5. Keep only the needed number of cuvettes outside the cooler box (one kit box at a time). The cuvette reagents should warm up to room temperature (18-25 °C) before measurement. Keep the rest of the reagent kit boxes and controls in the electronic cooler box.

5.2.6. 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 DCF13b (Appendix 1).

5.2.7. Prepare the DBS card and write the child ID and date of sample collection on it. Prepare a capillary and a plunger for collection of blood close to yourself so that they are easy to reach when the blood is ready to be collected. Prepare also the lancet so that you can reach it easily when the heel is cleaned and ready in your hand. Prepare the alcohol wipe and adhesive bandage for cleaning and covering the wound.

5.3. Collecting DBS and stool sample, and measuring CRP and Hb:

5.3.1. 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. The incision with the lancet may hurt for a brief moment and the caregiver should help in keeping the child still during the obtaining of the sample. Ask the caregiver to hold the child steady and preferably so that the child cannot see the lancet and the procedure. Hold the heel firmly and lower than the child's heart (so that gravity helps to pull blood to the heel).

5.3.2. Clean the medial or lateral side of the heel with an alcoholic wipe.

5.3.3. Use the clean lancet to make an incision on the cleaned site.

5.3.4. Let some blood make a droplet. Clean the first drop with the alcoholic wipe. Let the second drop develop.

DBS:

5.3.5. Apply pressure and let a large droplet develop.

5.3.6. Gently let the blood drop touch the filter paper circle and let the blood soak into it.

5.3.7. Repeat so that at least two circles are filled.

5.3.8. Leave the DBS drying horizontally (at least 3 hours) in room temperature using DRY RAK while keeping it away from direct sunlight. Do not stack the DBS or let it touch any other surfaces during the drying.

CRP + Hb:

5.3.9. Fill the capillary by putting the capillary's open end to the blood drop. The capillary is filled by itself. Wipe the capillary end with a sterile paper towel. Beware of air bubbles. If there is an air bubble in the capillary, take a new capillary or start over.

5.3.10. Put the capillary in the cuvette and push the blood into the cuvette buffer with the plunger.

5.3.11. Push on the lancet wound with a clean paper towel until the bleeding stops, put on an adhesive bandage and ask the caregiver to keep the area clean until the wound has healed.

5.3.12. Put the cap on the cuvette but make sure to not push in the inner blue part of the cap.

5.3.13. Push on "Measure" in the quick read go instrument. The lid will open automatically and guide you to put the cuvette in the measurement well. Put in the cuvette in the measurement well so that the barcode is facing towards you (forward toward the screen).

5.3.14. The measurement is automatic. Wait until the CRP and Hb results are seen on the screen.

5.3.15. Enter the CRP and Hb results in the sample collection form.

5.3.15.1. If the child is clearly ill, no study drug should be given, and they should be actively referred to the nearest health clinic.

5.3.15.2. If the child is asymptomatic or has mild respiratory symptoms, and the CRP is more than 50 or the Hb is less than 50, the child will be given the study drug and then actively referred to the nearest health clinic. Transportation will be ensured.

5.3.15.3. On the second visit if the Hb is less than 93 the child will be actively referred to the nearest health clinic and transportation will be ensured.

5.3.15.4. Upon referral, a paper copy of the instruction to health clinics (Appendix 4) will be given to the caregiver to show at the clinic.

5.3.16. Dispose of clinical waste and clean the surfaces.

See also less detailed pictorial instructions in Appendix 3 and a video of the whole process behind the link:
https://www.youtube.com/watch?v=QDprwMdv7y4)

Stool sample:

5.3.17. Ask the caregiver for the stool sample that was collected at home using the stool container they receive one day earlier. Put the barcode 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 (beginning with number 4, 4xxxxx) manually in the sample collection form. Put the stool sample in the cooler box for transport later to Kita/Bamako lab for processing and storage.

5.3.18. 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.

DBS packing:

5.3.19. Scan the DBS barcode into the tablet. When the DBS samples have dried for 3 hours, put each DBS card into their plastic zip lock bag with one desiccant packet to keep the DBS card dry in the bag during transport/storage. Ship in cool and dry environment, sun and heat deteriorate the DBS.

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 CRP+Hb+DBS 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*.

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) 13b-mechanistic sub-study
Appendix 2	Sample logbook
Appendix 3	Brief instructions of CRP+Hb measurement pictorial
Appendix 4	Instructions to health clinics in case of a referral

9. Version history, authors and approvals

Version (date)	Edits to the SOP text (author)
0.1. (2022-03-01)	Rikhard Ihamuotila in consultation with Yuemei Fan, Per Ashorn, Laura Adubra, Ulla Ashorn
1.0. (2022-03-15)	Rikhard Ihamuotila in consultation with Yuemei Fan, Per Ashorn, Laura Adubra, Ulla Ashorn

Appendix 1: Data Collection Form (DCF) 13b-mechanistic sub-study

Version 0.1, April 11, 2021

Section Header	Question Text	Question Responses	Required
Form 13b — Biological Sample Collection-mechanistic sub-study	Instructions: Complete this form for targeted age group children.		
	Interviewer ID (study nurse ID)		Yes
	Child ID (child ID sticker)		Yes
A. VISIT INFORMATION	1. Date		Yes
	2. MDA round (Visit number)	4S-00 / 4S-14	Yes
	3. Sample collection place	Village central place/pop-up facility	
	4. Child age group	4-11 mo	Yes
B. SAMPLE COLLECTION	5. What samples collected?	Heel prick blood CRP | Heel prick blood Hb | Heel prick blood, blood spot | Stool	Yes
	6. Was a heel prick blood sample collected?	Yes | No	Yes
	6a. Record the result of CRP	CRP =	Yes
	6b. Record the result of Hb	Hb =	Yes
	7. How many blood spots were stored on filter paper card?	0 | 1 | 2	Yes
	7a. Identifier (barcode) of the filter paper card?		Yes
	8. Was a stool sample collected?	Yes | No	Yes
	8a. What time the whole stool sample was collected?		Yes
	8b. What date and time did the child pass the stool?		Yes
	8c. Identifier (barcode) of the stool 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.

QuikRead go® wrCRP & wrCRP+Hb

Brief instructions

1 Remove the foil cover from the cuvette. The liquid surface should lie between the two lines marked on the cuvette. Do not touch the clear flat surfaces in the lower part of the cuvette.

2 Fill the the orange striped capillary with the sample (10 µl) up to the white stopper. Ensure that there are no air bubbles in the capillary. Wipe away any residual sample from the outside of the capillary.

3 Place the sample (10 µl) into the buffer solution in the cuvette and dispense by pressing down the plunger. Make sure that the capillary is completely empty.

4 Close the cuvette tightly with wrCRP reagent cap. Do not press down the turquoise inner part of the cap. Once the sample has been added to the buffer the assay should be run within two hours.

5 Choose Measure on the display of the QuikRead go instrument.

6 Insert the cuvette in to the measurement well of the instrument. The bar code should be facing you. The display shows how the measurement is proceeding.

7 When the measurement is completed, the result is shown on the display and the cuvette will rise up from the measurement well automatically. With the QuikRead go wrCRP+Hb test the Hb result is shown below the CRP result.

Please consult the instructions for use before performing the test.

ORION DIAGNOSTICA

Appendix 4.

Referral of a study child to CSCOM

Date: _____

Child name: _____

Child weight: _____ kg

Home village: _____

LAKANA study ID: _____

Blood Hb concentration (g / dl): _____

Blood CRP concentration (mg / l): _____

Please be advised that members of the LAKANA study team have today seen this child and have measured her / his blood concentration of haemoglobin (Hb) and C-reactive protein (CRP). We have observed some abnormal laboratory values in these tests and are referring these children for your assessment. Below, we have listed the observed abnormality and our recommended action for the case (based on recommendations made in the Pocket Book of Hospital Care for Children, World Health Organization, 2013

☐ **Hb below 5.0 g / dl. The child has severe anaemia.**
Test for malaria with a rapid test. Treat for malaria, if the test is positive.
Perform blood transfusion if possible.
Follow other Malian guidelines for treatment of severe anaemia.

☐ **Hb between 5.0 g / dl and 9.2 g / dl. The child has moderate anaemia.**
Test for malaria with a rapid test. Treat for malaria, if the test is positive.
If malaria test is negative, provide iron syrup (3 mg / kg) for 3 months.
Advise the caregiver about good feeding practice.

☐ **CRP at or above 50 mg / l. The child has systemic inflammation and possibly an infection**
Test for malaria with a rapid test. Treat for malaria, if the test is positive.
Assess for symptoms indicative of symptoms that might warrant further diagnostics.
If malaria test is negative and no other clues, provide amoxycillin syrup (20 mg / kg twice daily), for 5 days.

We appreciate your collaboration and assistance to the child. If you have any questions, please do not hesitate to contact us.

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On behalf of the LAKANA study team
Prof. Samba Sow
Contact details