Standard Operating Procedures for the LAKANA trial
SOP Subvisit-09: Conducting sub-study visit 9
Version 1.0 (2023-07-05)

1. Purpose and overview:

This SOP provides instructions on how to conduct the sub-study visit 09, the 9th visit after the eight MDAs for the LAKANA secondary outcome sample formed from villages around the four selected health centers close to the city of Kita.

This SOP refers to SOPs Visit 01, Prep-02, Proc-01, Proc-04, Proc-05, Proc-06, Proc-11, Safety-01, and Data Collection Form (DCF) DCF01 (a,b,c,d,e,f), DCF03, DCF04, DCF05, DCF08, and DCF13/13a.

2. Applicability to and responsibilities of various staff members

Staff member	Responsibility
District Supervisor	- Plans date of entry in villages - Prepares data collector and nurse's material to be dispatched from Kita to CSCom. - Reports on the sub-study visit 09 to the CVD-Mali coordinator and to the Implementation Working Group (IWG)
Lab technician	- Prepares sample collection bags in which swabs, tubes with medium and labels for rectal and nasopharyngeal swab sample collection are included, prepare also other collection material to be dispatched from Bamako to Kita and to CSCom. - Handles samples in the Kita/Bamako lab.
Field supervisor	- Agrees on date of entry and equipment rental with village chief. - Works with nurse to transport study material from Kita to CSCom and store. - Packs data collectors' rucksacks - With study nurse, manages the disposal of study waste. - Oversees data transmission to server - Reports on the sub-study visit 09 to the district supervisor
Study nurse	- Packs sample collection material at CSCom and arranges transport to village. - Coordinates the setting up of the pop-up facility in village.

	- Completes DCF13 in tablet computer or in paper form 13a-AMR (Appendix 1). - Completes sample logbook (Appendix 2). - Collects biological samples from 4-14 mo. and 49-59 mo. old children and ensures samples are correctly labelled, transported to and deposited in Kita lab. - Malaria RDT test for 1-59 mo old children
Anthropometrist	- Takes anthropometric measurements. *Refer to SOP Proc 03 for detailed activities.* - Records anthro-related data in study app
Data collector	- Finds the compounds/households and requests permission to proceed for the sub-study visit 09 - Operates tablet computer - Provide child information and/or affix child ID sticker on LAKANA color card/cloth/hand - Records requests from community members and reports to field supervisor
Relais	- Helps data collector to identify compounds and households. - Guides of 4-14 mo. and 49-59 mo. old children and their caregivers to pop-up facility for AMR sample collection. - Guides selected household 1-59 mo old children and their caregivers to pop-up facility for malaria RDT test
Driver/ messenger	- Ensures the transport of nurse and sample collection material between CSCom and village. - Assists nurse in setting up pop-up facility. - Ensures the safe transport of sample collected in village to Kita lab.

3. Required materials

Item	Number	Specification
Identification Sticker for **1-11** mo. old infants	2 identical stickers per child	2 identical stickers (with QR code/ID): • One sticker to be affixed on **child health card.** • Second sticker to be affixed on the data collector's paper register.

Item	Number	Specification
Identification Sticker for **12-14** mo. and **49-59** mo. old children	2 identical stickers per child	2 identical stickers (with QR code/ID) to be affixed on **LAKANA card**.
LAKANA card - **YELLOW**	*As required*	Card for **4-5** mo. old infants.
LAKANA card – **BLUE**	*As required*	Card for **6-8** mo. old infants.
LAKANA card - **GREEN**	*As required*	Card for **9-11** mo. old infants.
LAKANA card – **PINK**	*As required*	Card for **12-14** mo. old children **Card where the child sticker will be affixed.**
LAKANA card - **VIOLET**	*As required*	Card for **49-59** mo. old children **Card where the child sticker will be affixed.**
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	1	The following questionnaire will be loaded: DCF02b, DCF13
DCF13d, hard copy Appendix 1	*As required*	The paper version DCF13a 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.
Sample bag	1 sample bag/participant *Number required to meet the daily target*	Each sample bag includes two smaller zip bags. The "Rectal" one includes 3 flocked swabs (Copan swab with 30mm breakpoint - 520CS01), one 2mL tube with 1mL Cary Blair media, one 2mL tube with 1mL DESS, and one empty 2mL tube. The "NP" one includes 1 nasopharyngeal flocked swab (Copan swab with 100mm breakpoint - 503CS01), one 2mL tube with 1mL

Item	Number	Specification
		STGG media. Each tube with barcode label sticker on it.
Extra bag of Flocked swabs (both rectal swabs and nasopharyngeal swabs)	*Extra number in case needed*	Each bag includes 20 flocked rectal swabs (labelled with "extra rectal swabs) or 20 flocked nasopharyngeal swabs (labelled with "extra NP swabs). To protect from the contamination if the swab in the sample bag touch other surface or sample is not collected properly and need to be collected again
Disposable gloves	*Number required to meet the daily target*	To protect personnel and prevent spread of pathogens and avoid contamination between samples
Baby wipe	1 wipe/participant *Number required to meet the daily target*	To wipe the child's bottom prior to rectal swab sample collection.
Cooler box	1	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	*Number required to meet the daily target*	For cleaning surfaces
Biohazard waste bag	1	
70% ethanol	1	500 ml
Refrigerator	1/CSCom	To store and maintain transport media at appropriate temperature (2-8°C) and prepare/store ice packs to be used for maintaining cooler boxes.
Instruments for anthropometric measurements	*As required*	*Refer to SOP Proc-03*
Malaria P.f./Pan RDT cassette, One Step, Home Health UK	1 / participant	Rapid test for detecting plasmodium falciparum (P.f.), P. vivax, P. ovale and P. malariae.
Prolance pediatric lancet	1 / participant	For making the skin incision to get blood for malaria RDT test. 1,2 mm, HTL-Strefa S.A.

Item	Number	Specification
Alcohol wipes	1 /participant *Number required to meet the daily target*	For cleaning the skin before malaria finger prick
Adhesive bandage/plaster	*1/participant*	Adhesive bandage 19x72 mm
Marker pen	*As required*	To write ID on child's hand

4. Definitions and general instructions

4.1. Definitions

4.1.1. District Supervisor: a LAKANA staff member coordinating trial activities at the district level. The district supervisor reports primarily to the study coordinator.

4.1.2. Field Supervisor: a LAKANA staff member responsible for coordinating data collection teams' activities. He is under the supervision of the district supervisor.

4.1.3. Data collector (DC): a LAKANA staff member collecting data at the compound, household, and individual level.

4.1.4. Lab technician: a LAKANA staff member based in Bamako (Bamako lab), and responsible for preparing sample collection bags and material for study nurse and handling specimens brought from villages.

4.1.5. Study nurse: a LAKANA staff member responsible for AMR and mechanistic sub-study data and sample collection.

4.1.6. Measurer/assistant measurer: LAKANA staff member responsible for taking anthropometric measurements and recording corresponding data in study app.

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

4.1.8. Eligible child for **AMR** study: child aged between **4-14 or 49-59 mo.** living in a household that consented to participate in the LAKANA AMR study.

4.1.9. Eligible child for **malaria surveillance** study (RDT): child aged **1-59 mo.** living in a selected household that consented to participate in the LAKANA malaria surveillance study.

4.1.10. Eligible child for the growth study: child aged 6-8 or 12-14 mo. living in a household that consented to participate in LAKANA Growth study.

NB: Consent to participate in LAKANA sub-studies is requested only once at the enrolment of the household. The consent given at the enrolment stage applies to all eligible children that will be found at the subsequent visits. The enrolled household representatives can decide to discontinue the study participation at any point without giving a reason.

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

4.1.12. 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.

4.1.13. Implementation Working Group (IWG): LAKANA investigators identified by project steering group to oversee day-to-day study activities.

4.2. General Instructions

4.2.1. At the visit 9 in a village, the DCs will first meet the village chief or his/her representatives and inform that the LAKANA staff will be conducting the study visit 9. Next visit for AMR sample collection will be 1 year after.

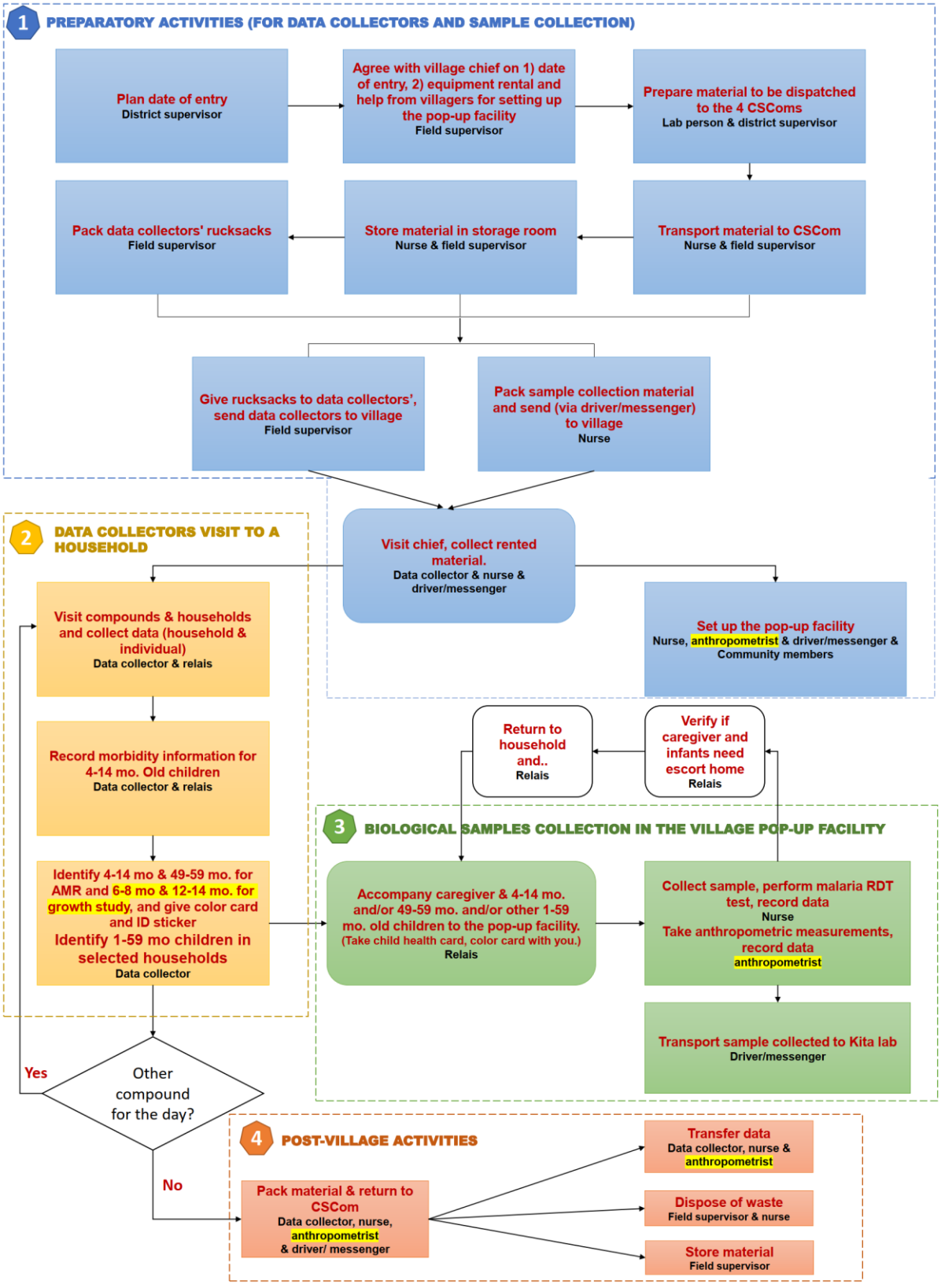
4.2.2. At the visit 9 in a village, there will be no distribution of the investigational drugs.

- The DCs will conduct interviews and record the vital status of household members and the Immunization and SMC-related information of eligible infants and **morbidity information for 4-14 mo children**. The DCs will also fill malaria survey form for selected household and identify eligible children for AMR, malaria RDT test and growth study.

  NB: malaria RDT test will be done for 1-59 mo children from households which are randomly chosen and DCs will be able to see from study App if the household is selected or not.

- The nurse will collect nasopharyngeal and rectal swabs from 4-14 mo and 49-59 mo children. The nurse will also perform malaria RDT test for 1-59 mo children from selected households, record results and provide a referral letter to the CSCom for those with positive results to receive treatment for malaria.
- The anthropometrist will take anthropometric measurements and record anthro-related data in study app.

4.2.3. The four main processes – 1) Preparatory activities (for data collectors and sample collection), 2) Data collectors visit to a household, 3) Biological samples collection, malaria RDT test and anthropometric measurements in a pop-up facility in the village, and 4) Post-village activities. Conducting sub-study visit 09 (visit 9S) are illustrated in the figure below and detailed instructions for each phase are provided in Section 5 Step-by-step procedures.



5. Step-by-step procedures

5.1. Agree with village chief:

5.1.1. Prior to entry in a village (~1 week), the field supervisor will agree with the chief of the village on the date of entry. At this occasion, the field supervisor will discuss the rental of some material (1 table + 3 chairs) and help from the community to set-up a pop-up facility for sample collection in the village.

5.2. Prepare material for a CSCom:

5.2.1. At the Bamako lab (~1 week prior to date of entry in village), the lab team will prepare the sample collection bags (*Refer to SOP Pro-04*) and material to be dispatched to the Kita district office and kept at 2-8°C. The malaria RDT test to be dispatched to the Kita district office and stored at 2-30°C.

5.2.2. The transport media needs to be aliquoted to 2 ml screw cap tubes before a village MDA. The laboratory technician will aliquot 1 ml Cary Blair transport media, 1 ml DESS and 1 ml STGG to 2 ml screw cap tubes and affix each tube with pre-printed unique barcode label sticker. S/he will prepare enough tubes in advance, store them (2-8°C) and test the medium 1 week before packing to make sure the medium is in good condition and not contaminated (*Refer to SOP Pro-04, Appendix 1-3*).

5.3. Transport material from Kita to CSCom and store:

5.3.1. The day before entry in a village, the nurse and field supervisor will pick up the material prepared and arrange the transportation to the CSCom.

5.3.1.1. The quantity of sample collection bags and material to be picked up will cover at least one week of activities per village.

5.3.1.2. During transportation, the sample collection bags will be kept at 2-8°C. The malaria RDT test will be kept at 2-30°C.

5.3.2. The nurse and field supervisor will store the material in the LAKANA storage room at the CSCom.

5.3.2.1. When going to the village, the nurse will keep the sample bags at 2-8°C in the electric cooler box. The nurse will also keep the malaria RDT test at 2-30°C.

5.3.3. The field supervisor will contact the data collectors assigned to the village and set an appointment at the CSCom on the morning of the date of entry in the village.

5.4. Entry in a village:

5.4.1. On the morning of the date of entry in a village, the field supervisor will prepare and distribute the rucksacks to the data collection team. The study nurse will pack the sample collection material needed for the day. The anthropometrist will pack the instruments needed for anthropometrics measurements.

5.4.2. The whole team will move together to the village. The data collector has his/her own transportation and a LAKANA driver/messenger will transport the nurse, the anthropometrist, the sample collection material and instrument for anthropometric measurements.

5.4.3. The field supervisor will visit the data and sample collection team to provide technical support, when needed. When on-site visits are not possible, the field supervisor will rely on regular phone calls.

5.4.4. On the first day of the visit 9 in a village, the DCs, nurses and anthropometrist will visit the chief or his/her representative and explain the visit:

5.4.4.1. Inform the representatives that no drugs will be distributed this time and that only interviews will be conducted, swab samples collected and malaria RDT and anthropometric measurements performed. The last AMR visit will be after one year.

5.4.4.2. Thank the village representatives for their time and support to LAKANA staff throughout the 2-year follow-up, and for the village participation in the trial. The DC will inform the village representatives that they will later be called to a meeting in which information on trial findings will be distributed.

5.4.4.3. At this encounter, the DC may also offer the village representatives a gift to acknowledge the village participation in LAKANA trial. The exact nature of the gift will be decided by the CVD-Mali coordination team, and it may vary during the LAKANA trial implementation.

5.4.5. The DCs will record any questions or comments the village representatives may have regarding LAKANA and will share the information with their field supervisor.

5.5. The DCs will thank the chiefs and members of compounds and households for their time and participation in the study. They will record any questions or comments the participants may have and report to the field supervisor.

5.6. The data collector accompanied by two Relais will start visiting compound/households.

5.7. The nurse, the anthropometrist and the driver/messenger and the community members will set-up the pop-up facility.

5.8. Data collection in household:

5.8.1. Like the previous MDA visits in the village, the DCs will visit the compounds and households enrolled in the trial and collect:

- **Vital status information of all members (DCF06)**
- **Immunization and SMC data for eligible infants (DCF03)**
- **Morbidity data for 4-14 mo children (DCF08)**
- **Maria survey form for selected household**

The differences with the MDA visits are that at visit 9:

- **No** drug will be distributed
- **No** new compounds or households will be enrolled in the study

5.8.2. LAKANA cards: The data collector will issue colored LAKANA cards to children eligible for AMR and growth Sub-study. The purpose of these colored

cards is to differentiate children's age-groups so that at the pop-up facility the nurse can identify what sample to collect and the anthropometrist can identify children whose anthropometic measurements should be performed according to the child's age group. In the household, the data collector will give different colored card to children for whom there is a household consent to participate in Sub-study:

- **Yellow** card to **4-5** mo. old infants
- **Blue** card to **6-8** mo. old infants
- **Green** card to **9-11** mo. old infants
- **Pink** card to **12-14** mo. old children
- **Violet** card to **49-59** mo. old children

5.8.3. ID stickers:

5.8.3.1. For **4-14** and **49-59 mo.** old children the data collector will affix the ID stickers on the child health card/LAKANA colored card. The data collector will instruct the caregiver to bring the colored card (that has the stickers) when visiting the pop-up health facility for nasopharyngeal and rectal swab sample collection, malaria RDT test and anthropometric measurements. For those 4-14 mo. children who have received ID stickers in previous MDAs, the DC will instruct the caregiver to bring the child health card with ID sticker and/or LAKANA colored card to pop-up facility.

5.8.3.2. For **1-59 mo.** old children from selected household in the malaria survey, the data collector will mark their hands with a marker pen to facilitate triage at the pop-up facility.

5.8.4. For all **4-14-mo. and 49-59 mo. old children** for AMR sample collection and all 1-59 mo. children from households selected to participate in the malaria community survey (for whom there is a household consent for participating in the sub-study), the Relais will accompany the caregiver and the children to the pop-up facility for sample collection, malaria RDT test and anthropometric measurements. The Relais will make sure the caregiver of each child has the LAKANA colored card/health card (if applicable), ID sticker (if applicable) and hand mark for malaria RDT test.

5.8.5. The data collector will continue with his activities in the households/compound (repeat step 5.8)

5.9. Data and Sample collection, malaria RDT test and anthropometric measurements at pop-up health facility

5.9.1. Every time a Relais will arrive at the pop-up facility with an eligible child for sample collection:

5.9.1.1. The nurse will open a new record (DCF13) on his/her tablet and start entering ID data.

- If the child age is younger than 14 mo., the ID will be found on the health card
- If the child age is between 49-59 mo. for AMR NPS and rectal swabs sample collection, the ID will be found on the LAKANA colored card.
- If the child age is 1-59 mo. and from households selected for malaria survey, the ID will be found on the child's hand marked with pen.
  NB: If entering the child ID information is not working, fill in the DCF13d-AMR paper form (Appendix 1). Study nurse will ONLY fill sample collection form DCF13d-AMR paper version WHEN the electronic DCF13 in the tablet is NOT working.

5.9.1.2. Based on the LAKANA colored card the child has or not, the nurse will select the corresponding age group on the tablet. By doing so, the data collection system will indicate the required samples to collect. The nurse will explain the sample collection procedure and give instructions to the caregiver.

5.9.1.3. The nurse will collect the rectal swabs and nasopharyngeal swab samples at Visit 09 (*Refer to SOP_Proc-05 and Proc-06*) and perform the malaria RDT test (Appendix 4: Instructions of malaria RDT test; 5.9.1.5 below) and record information on DCF13. The nurse will also provide a referral letter to the CSCom for those with positive malaria RDT results to receive treatment.

- The nurse will ensure the specimen storage in the appropriate temperature and condition in the cooler box. S/he will ensure that samples are correctly recorded and connected to the child ID – scan the barcode on the sample tubes to the tablet. Perform the malaria RDT test and record the results into the tablet.

  Note: When there is no barcode label or the barcode label cannot be scanned/recognized for the sample vial, the study nurse will handwrite the following information on the sample bag. The study nurse should also take a photo of child ID sticker.

  Barcode labels number for sub-study visit 09 starts from 900001.

  LAKANA-AMR, Visit 9
  Child ID (MUST be clear and recognizable)
  Child age (age group in months)
  Date of sample collection
  Name of Village

5.9.1.4. After samples are collected and information entered in DCF13, the nurse will fill the sample logbook for each sample collected (Appendix 2). The nurse will also keep the LAKANA colored card in a dedicated box. S/he will not give back the colored card to the caregiver.

5.9.1.5. Malaria RDT test

- The nurse will explain the purpose and process of the procedure and only little amount of blood is taken and 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.
- Allow the test, buffer to reach room temperature (15-30°C) prior to testing. Bring the test pouch to room temperature before opening it. After opening, use it as soon as possible.
- Place the cassette on a clean and level surface.
- Clean child finger with an alcohol wipe. Use a clean lancet to make an incision on the cleaned site.
- Use a pipette/disposal specimen dropper to transfer 5 µl of whole blood to the specimen well, then add the entire buffer and start the timer.
- Wait for the colored line(s) to appear. Read results at 10 minutes. Do not interpret the result after 20 minutes.
- Interpretation of results:

  POSITIVE: Two or three distinct colored lines appear.

  - P. falciparum or mixed malaria infection: one line appears in the control region, one line appears in Pan line region and one line appears in P.f. line region.
  - P. falciparum infection: one line appears in the control region, and one line appears in P.f. line region.
  - Non-falciparum plasmodium species infection : one line appears in the control region and one line appears in Pan line region.

  NEGATIVE: Only one colored line appears in the control region.

  INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test device. If the problem persists, discontinue using the test kit and contact your supervisor.

- The nurse will record the results and take a photo of the RDT results with tablet.

5.9.2. For children aged 6-8 mo and 12-14 mo, after collecting all samples and malaria RDT test, recoding all information in the app, the nurse will send them to the antheropometrist.

5.9.3. The anthropometrist will measure height, weight, MUAC, oedema and fill in the antheropometric form (DCF07) (*Refer to SOP_Proc-03*).

5.10. Sample transportation to Kita lab:

5.10.1. The nurse will coordinate with the driver/messenger the safe sample transportation to the Kita laboratory in appropriate time. *Refer to SOP Proc-05 (5.1.12) and SOP Proc-06 (5.1.16).*

5.11. Closing of the day:

5.11.1. After having reached the number of households/infants planned for the day, the LAKANA team will pack all the study material and return to the CSCom.

5.11.2. The data collector, the study nurse and the anthropometrist will transmit the data to the server.

5.11.3. The field supervisor and nurse will manage clinical waste. The nurse will put the remaining unused sample bags at 2-8°C in the refrigerator and store the malaria RDT test at 2-30°C.

5.11.4. The field supervisor will charge the tablet computers.

5.11.5. The field supervisor will report to the district supervisor on the study progress and on any issues that need to be addressed.

5.12. At the end of the visit 9 in a village, the DCs will thank the Relais for their time and assistance with LAKANA study activities. The DCs will record any questions or comments the Relais may have and report to the field supervisor.

5.13. The field supervisor will report to the district supervisor on the visit 9 and on any issues that needs to be addressed.

6. Occupational Safety Issues

6.1. The study nurse and the anthropometrist 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 change gloves before collecting nasopharyngeal swab after collecting rectal swab sample and perform malaria RDT test. S/he will change gloves after each study participant.

6.2. All concerned 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 recovery of microorganisms and storage.

6.3. The study nurse will dispose of all contaminated waste (gloves, papers, swab handles, etc.) into biohazard waste bags for incineration or disposal.

6.4. During 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 described in SOP-Safety 01.

7. Quality Assurance / Quality Control

The study nurse who will collect specimen will undergo practical training for rectal swab and nasopharyngeal swab sample collection, and for malaria RDT test. Study nurse will not be approved to collect the specimen and perform malaria RDT test until a supervising clinician has assessed their competency and signed off in the training log.

The anthropometrist who will perform anthropometric measurements should follow quality assurance / quality control in SOP_Proc-03_Performing anthropometric measurements.

8. Appendices and other related documents

Document number (Version)	Document content
Appendix 1	Data Collection Form (DCF) 13d-AMR
Appendix 2	Sample logbook
Appendix 3	Flowchart for Conducting the AMR Sub-study visit 9
Appendix 4	Instructions of malaria RDT test

9. Version history, authors and approvals

Version (date)	Edits to the SOP text (author)
Version 1.0 (2023-07-05)	Yuemei Fan, Laura Adubra, Dagmar Alber in consultation with CVD-Mali and IWG. Approved by LAKANA PSG.

Appendix 1: Data Collection Form (DCF) 13d-AMR

Form 13d: Biological Sample Collection

Version 0.1, 24 March 2023

Section Header	Question Text	Question Responses	Required
Form 13d — Biological Sample Collection	Instructions: Complete this form for targeted age group children.		
	Interviewer ID (filled in automatically)		Yes
	Child ID (filled in automatically?) *(Choose from a list?)*		Yes
A. VISIT INFORMATION	1. Date		Yes
	2. Visit number	Visit s9	Yes
	3. Child age in months and group	1-3 mo | 4-5 mo | 6-8 mo | 9-11 mo | 12-14 mo | 15-48 mo | 49-59 mo	Yes
B. SAMPLE COLLECTION	4. What samples collected?	Nasopharyngeal swab | Rectal swab | Finger prick blood	Yes
	5. How many rectal swabs were collected?	0 | 1 | 2 | 3	Yes
	5a. What time the rectal swabs were collected?		Yes
	5b. Identifier (barcode) of the first rectal swab in the empty tube - dry swab		Yes
	5c. Identifier (barcode) of the second rectal swab in Norgen Stool Nucleic Acid Collection Tube		Yes
	5d. Identifier (barcode) of the third rectal swab in Cary Blair media		Yes
	6. How many nasopharyngeal swabs were collected in STGG media?	0 | 1	Yes
	6a. What time the nasopharyngeal swabs were collected?		Yes
	6b. Identifier (barcode) of the nasopharyngeal swab		Yes
	7. Was a finger prick blood sample collected?	Yes | No	Yes

	7a. Record the result of malaria RDT	P. falciparum / non-falciparum plasmodium species / Negative / Invalid	Yes

Appendix 2: Sample logbook

Study name: LAKANA-AMR

Village name: _____

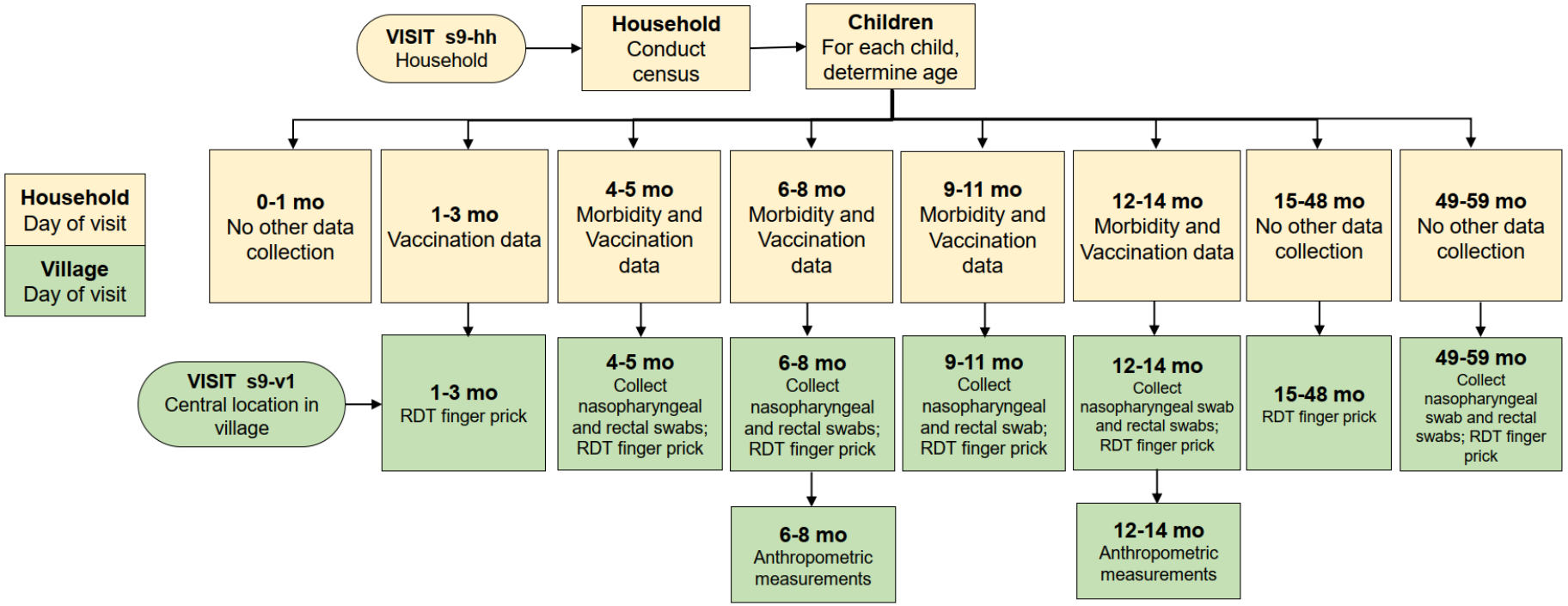
Study nurse (sample collector): _____

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

Appendix 3: Flowchart for conducting the AMR Sub-study visit 9



Appendix 4: Instructions of malaria RDT test

Malaria P.f. /Pan Rapid Test Cassette
(Whole Blood)
Package Insert

A rapid test for the qualitative detection of circulating antigens of P. falciparum (P.f.), P. vivax (P.v.), P. ovale (P.o.), and P. malariae (P.m.) in whole blood
For professional in vitro diagnostic use only

【INTENDED USE】

The Malaria P.f./Pan Rapid Test Cassette (Whole Blood) is a rapid chromatographic immunoassay for the qualitative detection of four kinds of circulating plasmodium falciparum(P. falciparum (P.f.), P. vivax (P.v.), P. ovale (P.o.), and P. malariae (P.m.)) in whole blood.

【SUMMARY】

Malaria is caused by a protozoan which invades human red blood cells.[1] Malaria is one of the world's most prevalent diseases. According to the WHO, the worldwide prevalence of the disease is estimated to be 300-500 million cases and over 1 million deaths each year. Most of these victims are infants, young children. Over half of the world's population lives in malarious areas. Microscopic analysis of appropriately stained thick and thin blood smears has been the standard diagnostic technique for identifying malaria infections for more than a century.[2] The technique is capable of accurate and reliable diagnosis when performed by skilled microscopists using defined protocols. The skill of the microscopist and use of proven and defined procedures, frequently present the greatest obstacles to fully achieving the potential accuracy of microscopic diagnosis. Although there is a logistical burden associated with performing a time-intensive, labor-intensive, and equipment-intensive procedure such as diagnostic microscopy, it is the training required to establish and sustain competent performance of microscopy that poses the greatest difficulty in employing this diagnostic technology.
The Malaria P.f./Pan Rapid Test Cassette(Whole Blood) is a rapid test to qualitatively detect the presence of P. falciparum - specific HRP-II and four kinds of circulating plasmodium falciparum(P. falciparum (P.f.), P. vivax (P.v.), P. ovale (P.o.), and P. malariae (P.m.)). The test utilizes colloid gold conjugate to selectively detect P.f-specific and Pan-malarial antigens (P.f., P.v., P.o. and P.m.) in whole blood.

【PRINCIPLE】

The Malaria P.f. /Pan Rapid Test Cassette(Whole Blood) is a qualitative, membrane based immunoassay for the detection of P.f., P.v., P.o. and P.m. antigens in whole blood. The membrane is pre-coated with anti-HRP-II antibodies and anti-Aldolase antibodies. During testing, the whole blood specimen reacts with the dye conjugate, which has been pre-coated on the test cassette. The mixture then migrates upward on the membrane by capillary action, reacts with anti-Histidine-Rich Protein II (HRP-II) antibodies on the membrane on P.f. Test Line region and with anti-Aldolase antibodies on the membrane on Pan Line region. If the specimen contains HRP-II or Plasmodium-specific Aldolase or both, a colored line will appear in P.f. line region or Pan line region or two colored lines will appear in P.f. line region and Pan line region. The absence of the colored lines in P.f. line region or Pan line region indicates that the specimen does not contain HRP-II and/or Plasmodium-specific Aldolase. To serve as a procedure control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

【REAGENTS】

The test cassette contains anti-HRP-II of Plasmodium falciparum antibodies conjugated gold and anti-Plasmodium falciparum Aldolase antibodies conjugated gold and anti-HRP-II antibodies and anti-Aldolase antibodies coated on the membrane

【PRECAUTIONS】

- For professional in vitro diagnostic use only. Do not use after expiration date.
- For whole blood specimen use only. Do not use other specimens.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.
- Do not exchange or mix buffer and test cassettes from kits of different lot number.
- Caution must be taken at the time of specimen collection. Inadequate volume of specimen may lead to lower sensitivity.
- Be sure to add sufficient buffer to the cassette's sample well. Invalid result may occur if inadequate buffer is added.

【STORAGE AND STABILITY】

The kit can be stored at room temperature or refrigerated (2-30°C). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

【SPECIMEN COLLECTION AND PREPARATION】

- The Malaria P.f./Pan Rapid Test Cassette (Whole Blood) can be performed using whole blood.
- Both Fingerstick Whole Blood and Venipuncture Whole Blood can be used.
- To collect Fingerstick Whole Blood specimens:
  - Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
  - Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger
  - Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
  - Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
- Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature for prolonged periods. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. For long term storage, specimens should be kept below -20°C. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly for more than three times
- If specimens are to be shipped, they should be packed in compliance with federal regulations covering the transportation of etiologic agents.

【MATERIALS】

Materials Provided

- Test Cassettes
- Buffer
- Disposable specimen droppers
- Package insert

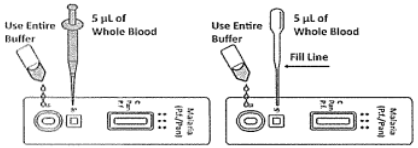
Materials Required But Not Provided

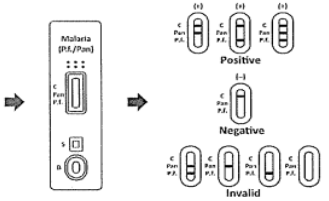
- Pipette and disposable tips (optional)
- Lancets (for fingerstick whole blood only)
- Specimen collection containers
- Timer

【DIRECTIONS FOR USE】

Allow the test, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible. Twist off the tab of the buffer vial without squeezing.
2. Place the cassette on a clean and level surface.
   - Use a pipette: To transfer 5μL of whole blood to the specimen well, then add the entire buffer and start the timer.
   - Use a disposal specimen dropper: Hold the dropper vertically; draw the specimen up to the Fill Line as shown in illustration below (approximately 5μL). Transfer the specimen to the specimen well, then add the entire buffer, and start the timer.
3. Wait for the colored line(s) to appear. Read results at 10 minutes. Do not interpret the result after **20 minutes.**





【INTERPRETATION OF RESULTS】

(Please refer to the illustration above)

POSITIVE:* Two or Three distinct colored lines appear.
P. falciparum or mixed malaria infection: one line appears in the control region, one line appears in Pan line region and one line appears in P.f. line region.
P. falciparum infection: one line appears in the control region, and one line appears in P.f .line region.
Non-falciparum Plasmodium species infection: one line appears in the control region and one line appears in Pan line region.
***NOTE:** The color intensity of P.f. or Pan Test lines may vary depending on the concentration of antigens, viz., HRP-II or Aldolase present in the specimen.
NEGATIVE: Only one colored line appears in the control region.
INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

【QUALITY CONTROL】

Internal procedural controls are included in the test. A colored line appearing in the control region (C) is an internal procedural control. It confirms sufficient specimen volume and correct procedural technique.
Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

【LIMITATIONS】

1. The Malaria P.f./Pan Rapid Test Cassette (Whole Blood) is for in vitro diagnostic use only. This test should be used for the detection of P.f., P.v., P.o., P.m. antigens in whole blood specimens only. Neither the quantitative value nor the rate of increase in P.f., P.v., P.o., and P.m. concentration can be determined by this qualitative test.
2. The Malaria P.f./Pan Rapid Test Cassette (Whole Blood) will only indicate the presence of antigens of Plasmodium sp. (P.f., P.v., P.o., P.m.) in the specimen and should not be used as the sole criterion for the diagnosis of malaria infection.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of malaria infection.

【EXPECTED VALUES】

The Malaria P.f./Pan Rapid Test Cassette (Whole Blood) has been compared with traditional thick and thin blood films microscopic analysis. The correlation between the two systems is over 99.0%.

【PERFORMANCE CHARACTERISTICS】

Sensitivity

The Malaria P.f./Pan Rapid Test Cassette (Whole Blood) has been tested with microscopy on clinical samples. The results show that the sensitivity of the Malaria P.f/ Pan Rapid Test Cassette (Whole Blood) is >98% when compared to results obtained with microscopy.

Specificity

The Malaria P.f./Pan Rapid Test Cassette (Whole Blood) uses antibodies that are highly specific to Malaria P.f.-specific and Pan-malarial antigens in whole blood. The results show that the specificity of the Malaria P.f./Pan Rapid Test Cassette (Whole Blood) is >99.9%, when compared to results obtained with microscopy.

Method		Microscopy			Total Results
		Positive		Negative	
Malaria P.f./ Pan Rapid Test Cassette	Results	P. v.	P. f.		
	Positive	54*	85**	0	139
	Negative	1	0	500	501
Total Results		55	85	500	640

Comment: Blood Samples infected by Plasmodium falciparum (n = 85), Plasmodium vivax (n = 54) were included, as well as 500 malaria negative samples to be confirmed with microscopy.
Note: * There was one P. vivax sample to show a P.v. line and a P.f. line.
There were two P. falciparum samples that they both showed a P.v. line and a P.f. line.
Relative Sensitivity for P.f.-specific antigens: 85/85>99.9 %(95%CI***: 96.5%~100.0%)
Relative Sensitivity for P.v. antigens: 54/55=98.2% (95%CI***: 90.3%~100.0%)
Relative Specificity: 500/500>99.9% (95%CI***: 99.4%-100.0%)
Accuracy: (54+85+500)/ (54+85+1+500) =99.8% (95%CI***: 99.1%-100.0%)
*** Confidence Intervals

Minimum Detection Level

Type	Parasites/μL
P. falciparum	200
Plasmodimum non-*falciparum* species (*P. vivax*)	1500

Precision

Intra-Assay

Within-run precision has been determined by using 15 replicates of four specimens: a negative, a P.f. positive, a Pan positive and an P.f./Pan dual positive. The specimens were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 15 independent assays on the same four specimens: negative, a P.f. positive, a Pan positive and an P.f./Pan dual positive. Three different lots of the Malaria P.f./Pan Rapid Test Cassette (Whole Blood) have been tested using these specimens. The specimens were correctly identified >99% of the time.

Cross-reactivity

The Malaria Rapid Test Cassette (Whole Blood) has been tested by HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, Syphilis, HIV, HCV, H. Pylori, MONO, CMV, Rubella and TOXO positive specimens. The results showed no cross-reactivity.

Interfering Substances

The following potentially interfering substances were added to Malaria negative and positive specimens

Acetaminophen	20 mg/dL	Caffeine	20 mg/dL
Acetylsalicylic Acid	20 mg/dL	Gentisic Acid	20 mg/dL
Ascorbic Acid	2 g/dL	Albumin	2 g/dL
Creatin	200 mg/dL	Bilirubin	1g/dL
Oxalic Acid	60 mg/dL		

None of the substances at the concentration tested interfered in the assay.

【BIBLIOGRAPHY】

1. Bill MaConell, Malaria Laboratory Diagnosis. January 2001.
2. Cooke AH, Chiodini PL, Doherty T, et al, Comparison of a parasite lactate dehydrogenase-base immunochromatographic antigen detection assay with microscopy for the detection of malaria parasite in human blood samples. Am J Trop Med Hyp,1999, Feb: 60(2):173-2.

Index of Symbols

	Attention, see instructions for use		Tests per kit	EC REP	Authorized Representative
IVD	For in vitro diagnostic use only		Use by	2	Do not reuse
2°C 30°C	Store between 2-30°C	LOT	Lot Number	REF	Catalog #
	Do not use if package is damaged				

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CE

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Number: 145719000
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