# Study Specific Procedure	**SOP** No: 007 **Version No:** 2.0 **Supersedes:** 1.0 **Effective Date:**

Title: Blood Collection SOP

	NAME	SIGNATURE	DATE
PREPARER	Caroline Ogwang		
REVIEWING AUTHORITY	Molline Timbwa		
Q. A AUTHORITY	Aisha Bwika		
APPROVING AUTHORITY	Caroline Ogwang		

1.0 PURPOSE

This SSP describes the procedure and scheduling for bleeding of patients enrolled in FLACSAM main study and PK sub-study.

2.0 SCOPE / RESPONSIBILITY:

2.1 This SSP is applicable to the study clinicians, nurses and fieldworkers.

2.2 Designated study clinicians, nurses and fieldworkers will carry out this procedure.

2.3 Overall responsibility related to implementation and quality control of this SSP lies with the PI through the lead clinician

3.0 DEFINITIONS/ ABBREVIATIONS:

3.1 PK: Pharmacokinetics

3.2 SSP: Study Specific Procedure

4.0 MATERIALS NEEDED:

4.1 Sample bottles/tubes (heparinised tubes)

4.2 Alcohol swabs/ cotton wool and spirit

4.3 Scalp vein needles

4.4 Needles

4.5 Syringes

4.6 Gloves

4.7 Tourniquet

4.8 Sharps container

4.9 Biohazard waste container/bag

4.10 Adhesive bandages/ tape

4.11 Lab request form

4.12 Extension with a T

4.13 IV cannulas

4.14 Malaria RDT

5.0 METHODOLOGY

5.1 Preliminary Points for Taking Blood samples:

- Every participant enrolled in the trial will have admission and discharge blood samples collected as follows;

- At enrolment 0.5 ml in EDTA tube, 1.0 ml in EDTA tube (purple top) and 1.0 ml in sodium heparin (green top) for biomarkers of infection and pathogen detection at the end of the study will be taken together with routine blood samples (full blood count, glucose, biochemistry, blood gas analysis, malaria RDT (see appendix 1) and blood culture where applicable.).
- For the 120 participants enrolled in the PK study, an additional sample; 1 ml in lithium heparin (orange) will be taken at enrolment and at 2 additional PK time points (within the first 24 hours of enrolment).
- At discharge 0.5 ml blood in EDTA tube, 1.0 ml of blood in EDTA tubes (purple top) and 1.0ml of blood in sodium heparin (green top) for biomarkers of infection and pathogen detection at the end of the study.
- For each readmission, we shall collect 0.5 ml in EDTA tube, 1.0 ml of blood in EDTA tubes (purple top) and 1.0ml in sodium heparin tube (green top) for biomarkers of infection and pathogen detection at the end of the study. This sample will only be collected at admission.

Number of days after enrolment	**Blood Sample to be collected**
At admission	(2.5ml) blood—1.0 ml EDTA, 1.0 ml Sodium Heparin, 0.5ml EDTA, malaria RDT
At discharge	(2.5ml) blood--1.0 ml EDTA, 1.0 ml Sodium Heparin, 0.5ml EDTA
45 days	No blood sample
90 days	No blood sample
Readmission to hospital	2.5ml blood--1.0 ml EDTA, 1.0 ml Sodium Heparin, 0.5ml EDTA, malaria RDT
PK participants	**Blood Sample to be collected**
PK samples at admission	(2.5ml) blood—1.0 ml EDTA, 1.0 ml Sodium Heparin, 0.5ml EDTA 1.0ml-Baseline PK sample, 2 other samples within 24 hours

- For the PK study, staff should prepare at least 15 minutes in advance for every bleeding time point to draw PK samples as close to scheduled time as possible. Preparation must involve;
  - Blood collection items to include tubes, syringes, logs, PK sampling tool, CRF, laboratory request forms

- Label apex tubes for ease of aliquoting by lab staff (Mbagathi and Coast General sites); this is applicable for the PK,1.0 ml EDTA and 1.0 ml sodium heparin plasma samples.
- Check patency and integrity of sampling cannula and change the cannula in advance if required.

- Sampling delays do not constitute deviations. The exact time of blood collection should always be documented.

5.2 Venepuncture Procedure:

a) Where no cannula in situ

1. Ensure that all blood collection equipment is prepared and ready for use prior to blood collection
2. Welcome the guardian/parent and child to the phlebotomy room and confirm their identity by asking their name and checking that it matches the name on the consent form or other identification document. Confirm that the details on the specimen request form i.e. names and initials match those provided by the child's parent/guardian.
3. Explain the phlebotomy procedure to the parent/guardian and child to alleviate any anxiety and answer any questions that may be asked. Sit /lay the child on the examination couch. Younger children may be carried on their parent's/guardian's lap.
4. Identify arm or other appropriate location on the child to prick. You may place a pillow, for comfort, under a fully extended arm. Put the tourniquet on the arm, placing it 5-8cm (3- 4 inches) proximal to the suitable vein and tighten it to expose the vein.
5. Clean the skin with an alcohol swab/ cotton wool soaked in spirit in a circular motion moving from inwards going outwards and allow to dry. It is the action of drying that kills the bacteria on the skin.
6. Grasp the child's arm firmly using your thumb to draw the skin taut to anchor the vein. The needle should form a 15 to 30-degree angle with the surface of the arm. Insert the needle through the skin and into the lumen of the vein. Avoid trauma and excessive probing.
7. Draw required volume of blood in the syringe i.e. between 6 and 8 mls at admission for blood culture, T0 sample (for PK participants) and for determination of molecular

biomarkers of infection and pathogen detection. Subsequent bleeds for only PK samples will be 1 ml while that for further samples for determination of molecular biomarkers of infection and pathogen detection will be 2.5 ml (at discharge and readmission). Pass the blood to an assistant to transfer to tubes (see 5.2 (c) below).

8. Open the tourniquet, then gently withdraw the needle from the vein, immediately press the puncture site firmly with cotton wool/ gauze and ask the parent/guardian to press firmly on the puncture site.
9. Dispose the needle and its attachments into the sharps container. Do not attempt to recap needles.
10. Check the site of the venepuncture and if the bleeding has stopped then participant can leave the room. If the site is still bleeding, continue to apply pressure for a further 5 minutes.

b) Where cannula in situ

1. Wash and dry hands thoroughly or use a hand sanitizer. Wear clean gloves.
2. About 1ml of heparinised saline will be used to maintain patency of the cannula. This volume of initial blood from cannula should be withdrawn and discarded prior to sampling to avoid risk of a diluted blood sample
3. Draw required volume of blood into syringe using a connection tube i.e. between 6 and 8mls at admission for blood culture, T_0 sample (for PK participants) and for determination of molecular biomarkers of infection and pathogen detection. Subsequent bleeds for only PK samples will be 1 ml while that for further samples for determination of molecular biomarkers of infection and pathogen detection will be 2.5 ml (at discharge and readmission). Pass the blood to an assistant to transfer to tubes (see 5.2 (c) below).
4. If the cannula is required for subsequent PK sample collection secure well with tape and flush with 1ml heparinised saline.

c) After withdrawal of blood by either method

1. Dispose-off contaminated materials/supplies in designated containers.
2. Transfer required volume of blood into appropriate tubes and a drop of blood to the malaria RDT kit in the phlebotomy room. Mix well by gentle turning of tube up and

down between 8 and 10 times. Label the tubes with date of collection, the subject's ID number and appropriate PK time point codes.

3. Pack the PK sample with a pre-labelled apex tube into a zip-locked bag, together with the completed lab requisition form. 1 aliquot will be saved in the apex tubes from the primary sample.
4. Deliver PK samples promptly to the laboratory for sample processing. It is recommended that samples are shipped to the lab immediately after collection so that PK samples are processed within 30 minutes of collection and the other samples within 1 hour of collection.
5. Document difficult draws in the requisition form for use in interpreting spurious results. The phlebotomist should not make more than 3 attempts. The 4th attempt should be made by another phlebotomist.
6. The 0.5ml EDTA sample will be frozen at -80^{0}C at in the same tube as whole blood while the 1.0 ml EDTA sample and 1.0 ml sodium heparin sample will be centrifuged and the plasma samples frozen at -80^{0}C. All these should be processed within 1 hour of collection.

NOTE:

- If a blood draw is not possible through an indwelling intravenous cannula, a small-gauge butterfly needle and syringe will be preferred over a needle and syringe for obtaining PK blood samples from infants and young children.
- The femoral vein should be avoided as much as possible for blood draws (i.e. research). The level of risk associated with this approach is significantly high to allow normal use for research purposes. Instead, femoral vein should be used whenever required in pursuit of clinical care for the patient. For research the antecubital fossa and any reasonably accessible or easily visible peripheral vein should be used.
- Tubes with additives must be fully mixed by gentle turning up and down. Erroneous test results may be obtained when the blood is not thoroughly mixed with the additive.
- If samples cannot be shipped immediately to the designated labs for processing/analysis they should be handled as follows:

- Blood culture specimens can be stored at room temperature but must reach the lab within 24 hours for processing
- The plasma (from the 1.0 ml EDTA sample and 1.0ml Sodium heparin sample) and serum (from the 1ml lithium heparin sample) aliquots after centrifugation will be frozen in a -20^{0}C/ -80^0 freezer for later analysis. The whole blood 0.5 ml EDTA sample will also be frozen in a -20^{0}C/ -80^0 freezer.

Blood should NEVER be poured from one tube to another since the tubes have different additives or coatings.

5.3 Safety and Infection Control:

- Consider all human biological material to be a biohazard and handle according to the Local Health and Safety rules (refer to KEMRI health and safety policy& safety and accident reporting section), using universal precautions.
- In case of pricks/work related injury refer to the safety and accident reporting SSP.

5.4 Schedule of phlebotomy

- Phlebotomy will be done as per the assigned time point for PK sampling and as per the study visit schedule.

5.5 Training

- All the field workers, study nurse/s and clinicians will be trained on the blood collection SSP.

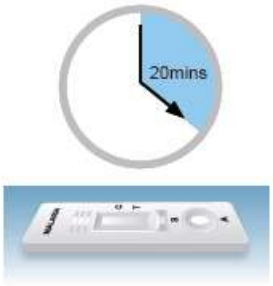
6.0 APPENDICES

Appendix 1 -Malaria RDT test

1. Add 5 $\mu\ell$ of whole blood into the 'S' well.

2. Add 60$\mu\ell$ assay buffer solution (3 drops for vial type or 2 drops for bottle type) into the "A" well.

3. Start a timer



4. Read result in 20 minutes.

7.0 REFERENCES:

7.1 FLACSAM protocol

8.0 DOCUMENT CHANGE HISTORY

Version Table:

Version 1.0: Title: **Blood collection SOP**	Dated: **13th Jul 2017**	SOP No. **007**	No. Pages: **8**
Version 2.0: Title: **Blood collection SSP**	Dated: **26th Nov 2019**	SOP No.: **007**	No. Pages: **9**
Version 3.0: Title:	Dated:	SOP No.:	No. Pages:

SSP Review and Updating Logs

DATE	NAME OF REVIEWER	SIGNATURE	REASON FOR REVIEW
26/11/2019	Molly Timbwa		• Periodic SOP review • Adopted the SSP template.

SSP AWARENESS LOG

I, the undersigned below, hereby confirm that I am aware that the accompanying SSP is in existence from the date stated herein and that I shall keep abreast with the current and subsequent SSP versions in fulfilment of Good Clinical Practice (GCP).

Number	Name	Signature	Date (dd/mmm/yyyy)
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