Molecular Testing for Malaria Standard Operating Procedure (SOP)

Collection of blood on filterpaper

Document ID: SOP-01

Developed by:

[Logos of the collaborating institutions]
Molecular Testing for Malaria: Overview of Standards

A number of consensus-adopted standards have been used in the development of this document. These include:

1. Recommended Genotyping Procedures (RGP) to identify parasite populations (World Health Organization, 2007).
3. Collecting, processing, and handling venous, capillary, and blood spot samples, (Program for Appropriate Technology in Health (PATH), 2005)
4. CLSI standard for blood collection on filter paper for newborn screening programs (Clinical and Laboratory Standards Institute, 2007).

In addition to the above standards, this document includes step by step procedures adapted from the Centers for Disease Control and Prevention and World Health Organization HIV Rapid Test Training package (Centers for Disease Control and Prevention, 2006).
**Signature Page**

By signing this page, staff members providing malaria recrudescence versus reinfection testing confirm they have read this SOP and guarantee to implement the procedures contained within.

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<th>Designation/affiliation</th>
<th>Signature</th>
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**Version History**

This SOP may be adapted to suit the particular needs of the individual laboratory. It is important to bear in mind that the purpose of an SOP is to ensure testing quality and result reproducibility.

<table>
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1. **Scope**

This SOP describes the method of collecting whole blood via finger stick for preparation as a Dried Blood Spot (DBS).

2. **Abbreviations**

DBS  Dried Blood Spot  
ml  millilitre  
PCR  Polymerase chain reaction  
SOP  Standard Operating Procedure  
µL  microlitre

3. **Personnel qualifications**

3.1. **Medical fitness**

Occupational health programs should be in place to monitor/address staff vaccinations and deal with exposures to potentially infected materials.

3.2. **Education and training**

Training must be given on the following topics:

- Hand washing;
- Wearing and use of personal protective equipment and clothing;
- Handling of potentially infectious materials;
- Prevention of incidents and steps to be taken by workers in case incidents (including biological, chemical, electrical and fire hazards) occur;
- Procedures;
- Waste management;
- Impact of results for patient management and research.

Training must be provided:

- When a new staff member takes up post;
- Annually;
• When there is a change in conditions or best practices.

4. Procedure

4.1. Principle

The collection and transportation of a high quality sample is critical in assuring accurate test results.

Capillary blood is collected from the finger of a patient and dried on an appropriate filter paper or card. High quality DBS samples, when stored correctly, have a prolonged shelf life. DBS samples are easily transported to the laboratory for testing.

4.2. Samples

Not applicable as this SOP details sample collection.

4.3. Required Materials and Equipment

Retractable sterile lancets (tip size less than 2.4 mm for adults, 2.0 mm for children and 1.5 mm for infants)

Scissors

Alcohol wipes containing 70% isopropyl alcohol

Sterile gauze pads

Disposable gloves (latex, vinyl, nitrile)

Eye protection

Sharps waste container (single use, combustible)

Filter paper or Guthrie cards

  *NB. Always use high quality blood collection paper: 903 or 2992. Poor quality paper can adversely affect test results.*

Small, clean plastic bags for storage of individual filter papers

Large plastic zipper-close bags

Desiccant packs
4.4. Procedural steps

Important points to remember:

- Wash hands between each patient.
- Ensure you have all necessary supplies in place before beginning the finger stick process.
- Remember to reassure the patient and explain the procedure thoroughly.
- Be careful to closely follow the recommendations for choosing the finger stick site.
- Always wear gloves when handling, labeling and cutting filter paper.
- A new filter paper is used for collection of each sample (and for each time point).

1. Print out a sample log sheet (in duplicate) and gather all required supplies.
2. Label the duplicate worksheets with the patient ID, sample ID, date and type of sample (e.g. pre/post day of therapy) using a ballpoint pen.
3. Prepare the filter papers as detailed below:

<table>
<thead>
<tr>
<th>Preparation of filter papers</th>
<th>5 cm</th>
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<tbody>
<tr>
<td>- Always wear gloves.</td>
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<tr>
<td>- Clean the scissors with 70% alcohol and dry.</td>
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<tr>
<td>- Cut a square of filter paper approximately 5 cm x 5 cm.</td>
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<tr>
<td>- Label the top of each filter paper twice (once on the back and once</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Preparation of filter papers.
on the front) with the patient ID/sample ID, date and type of sample (e.g. pre/post day of therapy) using a pencil, ballpoint or marker pen (see figure 1).

- Once labeled, fold filter paper as indicated by the dotted line (figure 1).

### Preparation of Guthrie cards

- Always wear gloves.

- Label each card twice with the patient ID/sample ID, date and type of sample (e.g. pre/post day of therapy) using a pencil, ballpoint or marker pen (see figure 2).

- Once labeled, fold filter paper backwards as indicated by the dotted line (figure 2).

![Figure 2. Preparation of Guthrie cards.](image)

4. Examine the patient’s fingers to identify the best location for the finger stick:

- Do not use the tip or centre of the finger.

- Avoid the side of the finger where the bone is closer to the surface.

- Avoid the 2\textsuperscript{nd} (index) finger as the skin tends to be thicker.

- Avoid the 5\textsuperscript{th} (small) finger as it tends to have less soft tissue overlying the bone.

- Avoid puncturing a finger that is cold, cyanotic (appears blue or extremely
5. Position the patient (lying down or sitting) and hyperextend the patient’s arm.  
   
   NB. Ensure the patient’s arm is kept to low in order to maintain blood flow.

6. Massage the finger to increase blood flow by gently squeezing from hand to fingertip 5-6 times.  
   
   NB. Excessive squeezing can cause concentration of tissue fluids which can adversely affect test results.

7. Clean the finger tip using an alcohol wipe and dry with a piece of gauze.

Figure 4. Clean finger with alcohol wipe. Photo taken from (Centers for Disease Control and Prevention, 2006).
8. Remove the sterile retractable lancet from its packaging, grasping it between thumb and forefinger.

   NB. Refer to manufacturer’s instructions when using another finger puncture device.

9. Firmly press the lancet to make a skin puncture just off the centre of the finger pad (see figure 1).

   ![Figure 1. Skin puncture](https://example.com/figure1.jpg)

   NB. The puncture is made perpendicular to the ridges of the fingerprint to prevent blood running along the ridges.

10. Wipe away the first drop of blood with a sterile gauze or cotton ball.

   ![Figure 2. Remove the first drop of blood](https://example.com/figure2.jpg)
**NB. The first drop of blood tends to contain excess tissue fluid which can adversely affect sample quality.**

11. Hold the finger down over the filter paper or first circle on the Guthrie card.  
*NB. Do not touch the circle.*

12. When a full hanging drop of blood is formed, gently touch the drop to the filter paper or inside the first circle.

   - Blood should only be applied from one side of the paper and appear as an even, uniform layer.
   - Do not layer blood drops on top of one another.

### Specific instructions applicable to filter paper collection

- Apply 3 drops of blood to the filter paper.

### Specific instructions applicable to Guthrie card collection

- Absorb the blood directly from the collection site onto the paper while watching the circle to ensure that it completely fills.
- If needed, continue to apply blood drops onto open areas of the first circle until it is completely covered.
- Fill the first circle completely before going onto the second circle and so on.

13. Once there is an adequate amount of blood on the filter paper, apply a gauze pad or cotton ball to the puncture site until the bleeding stops. It may be necessary to apply an adhesive bandage/plaster.
14. Air dry the samples for 3-4 hours on a flat, nonabsorbent surface (a stackable drying rack is ideal).

   *NB. Do not air dry samples for less than 3 hours. Do not expose samples to direct sunlight or extreme temperature or humidity. Do not heat-assist the samples to dry.*

15. Place the filter papers in individual plastic bags together with an individual desiccant pouch. If using Guthrie cards, 10-15 may be packed per bag together with at least 5 individual desiccant pouches.

   *NB. If samples are to be stored prior to transportation, they should be kept at +4 °C. For long term storage (over 90 days), samples must be stored at -20 °C*.  

<table>
<thead>
<tr>
<th>Sample Transportation</th>
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<tr>
<td>16. Enclose the completed worksheet with each batch of samples (retain one copy for receiving results).</td>
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<tr>
<td>17. Samples should be transported to the laboratory by the fastest means possible.</td>
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<td>It is recommended that samples be transported in a cooler (with ice packs) to protect against high temperatures and sunlight.</td>
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<tr>
<td><em>NB. When samples have been stored at +4 °C or -20 °C, they must be removed</em></td>
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</table>

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1 Samples may also be stored with regular refreshing of desiccant packs in a cool dark place if there is no freezer space. However, quality cannot be guaranteed for long periods.
5. Quality Control

It is critical to ensure the DBS sample is of the highest quality possible. Poor quality samples will lead to poor quality results. DBS sample quality control requires visual analysis of samples.

Always verify patient and sample ID on the specimen with that of the worksheet before sending to the testing laboratory.

The following samples are of not appropriate for testing:

- Specimens of insufficient quantity for testing;
- Specimens of insufficient quantity for testing;
- Specimen not dried before mailing;
- Specimen appears supersaturated;
- Specimen appears diluted, discoloured or contaminated;
- Specimen exhibits serum rings;
- Specimen appears clotted or layered;

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2 Adapted from CDC HIV Rapid Test Training “DBS Collection” (Centers for Disease Control and Prevention, 2006)
• There is no blood;

A high quality DBS sample should look like this (may not be as red in colour):

6. **Procedure limitations**

It can be difficult to obtain an adequate volume of blood from some patients. Keeping the finger at a level below the elbow and massaging the hand/finger should help.

7. **Interpretation and Reporting of Results**

Varies according to local protocol employed.

8. **Safety Precautions**

Always practice universal precautions (treat all patient specimens as potentially infectious material):

• Wear good quality, single-use, disposable medical examination gloves.
• Wear a laboratory coat or gown.
• Wash hands after removal of gloves.

Dispose of medical waste in the appropriate manner:

• Contaminated sharps (including lancets) must be disposed of immediately after use into a proper sharps waste container.
• Spills should be cleaned using 10% bleach.
9. Bibliography


Appendices
Appendix A – Bench Top Reference

1. Print out a sample log sheet (in duplicate) and gather all required supplies.
2. Label the duplicate worksheets.
3. Prepare the filter paper and record patient/sample details as shown in the SOP.
4. Examine the patient’s fingers to identify the best location for the finger stick:
   - Do not use the tip, side or centre of the finger.
   - Avoid the 2nd (index) and 5th (small) fingers.
   - Avoid puncturing a finger that is cold, swollen, scarred, calloused or covered with a rash.
5. Position the patient (lying down or sitting) and hyperextend the patient’s arm.
6. Massage the finger to increase blood flow by gently squeezing from hand to fingertip 5-6 times.
7. Clean the finger tip using an alcohol wipe and dry with a piece of gauze.
8. Remove the sterile retractable lancet from its packaging, grasping it between thumb and forefinger.
9. Firmly press the lancet to make a skin puncture just off the centre of the finger pad.
10. Wipe away the first drop of blood with a sterile gauze or cotton ball.
11. Hold the finger down over the filter paper or first circle on the Guthrie card.
12. When a full hanging drop of blood is formed, gently touch the drop to the filter paper or inside the first circle.
13. Once there is an adequate amount of blood on the filter paper, apply a gauze pad or cotton ball to the puncture site until the bleeding stops.
14. Air dry the samples for 3-4 hours on a flat, nonabsorbent surface (a stackable drying rack is ideal).
15. Place the filter papers in individual plastic bags / envelopes together with an individual desiccant pouch.
16. Enclose the completed worksheet with each batch of samples (retain one copy for receiving results).

17. Samples may be transported to the testing laboratory under ambient conditions.
### Appendix B – DBS Sample Collection Worksheet

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