

Molecular Testing for Malaria Standard Operating Procedure (SOP)

Collection of blood on filterpaper

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Developed by:



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 (RGPs) to identify parasite populations (World Health Organization, 2007).
2. Filter paper preparation (SOP), (Worldwide Antimalarial Resistance Network, 2010)
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).

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.

Version number	Revision(s) & reason for amendment	Date of approval	Approved by (lab supervisor / manager)
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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

Humidity indicators

Pens

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:

Preparation of filter papers

- Always wear gloves.
- Clean the scissors with 70% alcohol and dry.
- Cut a square of filter paper approximately 5 cm x 5 cm.
- Label the top of each filter paper twice (once on the back and once

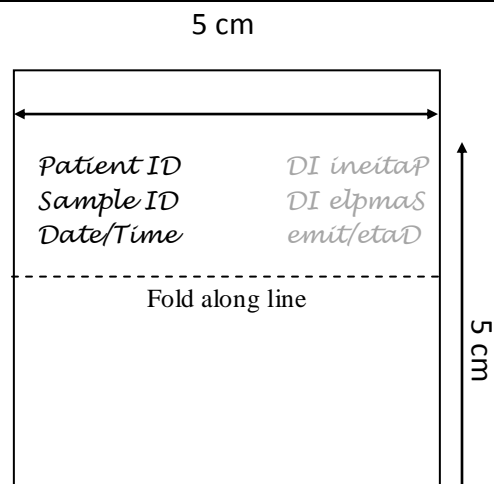


Figure 1. Preparation of filter papers.

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).

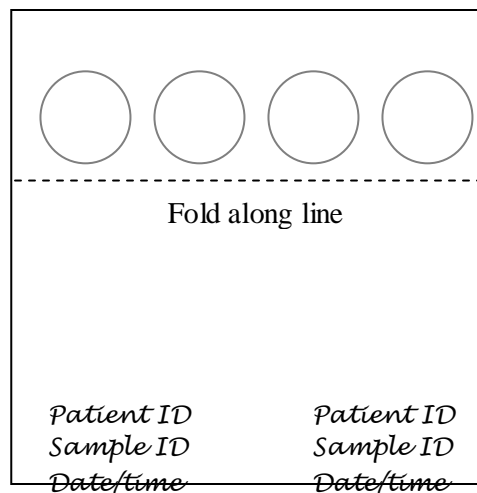


Figure 2. Preparation of Guthrie cards.

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 2nd (index) finger as the skin tends to be thicker.
- Avoid the 5th (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

pale in colour), swollen, scarred, calloused or covered with a rash.

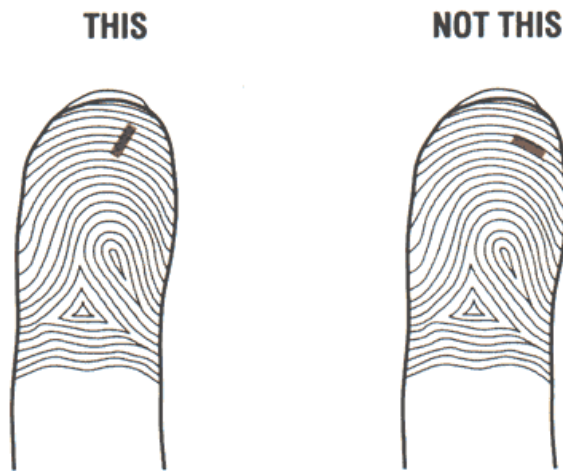


Figure 3. Correct placement of lancet for finger stick collection. Taken from (Program for Appropriate Technology in Health (PATH), 2005).

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).

NB. Drying the finger is important in order to obtain a blood drop at the puncture site.

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. Photo taken from (Centers for Disease Control and Prevention, 2006).

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. Photo taken from (Centers for Disease Control and Prevention, 2006).

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.



Figure 3. Apply gentle pressure to the puncture site. Photo taken from (Centers for Disease Control and Prevention, 2006)

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 °C¹.

Sample Transportation

16. Enclose the completed worksheet with each batch of samples (retain one copy for receiving results).

17. Samples should be transported to the laboratory by the fastest means possible. It is recommended that samples be transported in a cooler (with ice packs) to protect against high temperatures and sunlight.

NB. When samples have been stored at +4 °C or -20 °C, they must be removed

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 can not be guaranteed for long periods.








from cold storage, allowed to reach room temperature (in the closed bag) and the old desiccant pouches discarded and replaced prior to transportation.

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 testing²:

• 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;	

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

- Calderaro, A., Piccolo, G., Perandin, F., Gorrini, C., Peruzzi, S., Zuelli, C., et al. (2007). Genetic polymorphisms influence Plasmodium ovale PCR detection accuracy. *Journal of Clinical Microbiology*, 45(5), 1624-1627.
- Cattamanchi, A. A., Kyabayinze, D., Hubbard, A., & Rosenthal, P. J. (2003). Distinguishing recrudescence from reinfection in a longitudinal antimalarial drug efficacy study: comparison of results based on genotyping of msp-1, msp-2, and glurp. *American Journal of Tropical Medicine*, 68(2), 133-139.
- Centers for Disease Control and Prevention. (2006). *Blood collection - finger prick*. Retrieved August 17, 2010, from CDC HIV Training: <http://wwwn.cdc.gov/dls/ila/hivtraining/trainersguide/pdf/presentations/Module8Presentation.pdf>
- Clinical and Laboratory Standards Institute. (2007). Blood collection on filter paper for newborn screening programs; approved standard - fifth edition. *CLSI document LA4-A5*, 27(20).
- Falk, N., Maire, N., Sama, W., Owusu-Agyei, S., Smith, T., & Beck, H.-P. a. (2006). Comparison of PCR-RFLP and Genescan-based genotyping for analyzing infection dynamics of Plasmodium falciparum. *American Journal of Tropical Medicine.*, 74(6), 944-950.
- Färnert, A., Arez, A. P., Babiker, H. A., Beck, H. P., Benito, A., Björkman, A., et al. (n.d.).
- Färnert, A., Arez, A. P., Babiker, H. A., Beck, H. P., Benito, A., Björkman, A., et al. (n.d.). Genotyping of Plasmodium falciparum infections by PCR: a comparative multicentre study. *Trans R Soc Trop Med Hyg.*, 95.
- Kyes, S., Craig, A. G., & Marsh, K. a. (1993). Plasmodium falciparum: a method for the amplification of S antigens and its application to laboratory and field samples. *Experimental Parasitology*, 71, 473-483.

- Nakeesathit, S., Pagomrat, W., Tanomsing, N., & Hanchana, S. a. (2001). *DNA Extraction*. Bangkok: Mahidol Oxford Tropical Medicine Research Unit.
- Plowe, C. V., Djimde, A., Bouare, M., & Doumbo, O. a. (1995). Pyrimethamine and proguanil resistance-conferring mutations in *Plasmodium falciparum* dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa. *American Journal of Tropical Medicine and Hygiene.*, *52*, 565-568.
- Program for Appropriate Technology in Health (PATH). (2005). *RBP-EIA: collecting, processing, and handling venous, capillary, and blood spot samples*. Seattle: PATH.
- QIAGEN. (2007). *QIAamp DNA mini and blood mini handbook Second edition*. QIAGEN.
- Rubio, J. M., J., R. P., L., B. M., Garcia, M., M., M., & I., E. M. (1999). Semi-nested multiplex polymerase chain reaction for detection of human malaria parasites and evidence of *Plasmodium vivax* infection in Equatorial Guinea. *American Journal of Tropical Medicine and Hygiene.*, *60*, 183-187.
- U.S. Department of Health and Human Services. (2007). *Biosafety in microbiological and biomedical laboratories. 5th*. Washington, District of Columbia, USA: U. S. Government Printing Office.
- U.S. Department of Health and Human Services. (2007). *Biosafety in microbiological and biomedical laboratories (5th ed.)*. Washington, District of Columbia, USA: U. S. Government Printing Office.
- Watcharee, P., Naowarat, T., Supatchara, N., & Sarun, H. a. (2009). *Gel Electrophoresis*. Bangkok: MORU.
- World Health Organization. (2007). *Methods and techniques for clinical trials on antimalarial drug efficacy: genotyping to identify parasite populations*. Amsterdam: WHO.
- World Health Organization. (2007). *Recommended Genotyping Procedures (RGPs) to identify parasite populations*. Amsterdam: Medicines for Malaria Venture and World Health Organization.

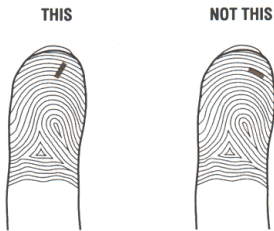
World Health Organization. (2008). *Consultation on Technical and Operational Recommendations for Clinical Laboratory Testing Harmonization and Standardization*. Geneva: World Health Organization.

Worldwide Antimalarial Resistance Network. (2010). *Filter paper preparation v1.0 (SOP ID: MOL03/CLIN06)*. Worldwide Antimalarial Resistance Network (WWARN).

Zwetyenga, J., Rogier, C., Tall, A., Fontenille, D., Snounou, G., & Trape, J.-F. a.-P. (1998). No influence of age on infectious complexity and allelic distribution in *Plasmodium falciparum* infections in Ndiop, a Senegalese village with seasonal, mesoendemic malaria. *American Journal of Tropical Medicine and Hygiene.*, 59(5), 726-735.

Appendices

Appendix A – Bench Top Reference

<ol style="list-style-type: none">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.	<ol style="list-style-type: none">4. Examine the patient's fingers to identify the best location for the finger stick:<ul style="list-style-type: none">• 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.	
<ol style="list-style-type: none">5. Position the patient (lying down or sitting) and hyperextend the patient's arm.	<ol style="list-style-type: none">6. Massage the finger to increase blood flow by gently squeezing from hand to fingertip 5-6 times.	<ol style="list-style-type: none">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

Number	Staff Initials	Patient ID	Sample ID	Date Taken	Time Taken	Comments
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						