

## Filter Paper Preparation v1.2

Procedure



**Molecular Module**

**WorldWide Antimalarial Resistance Network (WWARN)**



**Suggested citation:** WWARN. 2010. Filter Paper Preparation v1.2. WWARN Procedure.

**Procedure ID:** MOL03/CLIN06

**This procedure was developed by:**

Molecular Module, WWARN

Clinical Module, WWARN

#### **Version History**

<b>Version number</b>	<b>Revision(s) &amp; reason for amendment</b>	<b>Release date</b>
1.2	Changes to Materials and Equipment and Procedure	
1.1	Changes to template	27/10/2010
1.0	Creation of procedure	27/07/2010

**For more information, contact:**

molecular@wwarn.org

WorldWide Antimalarial Resistance Network (WWARN)

[www.wwarn.org](http://www.wwarn.org)

## Contents

1. Purpose .....	4
2. Scope .....	4
3. Abbreviations .....	4
4. Duties and Responsibilities .....	4
5. Materials and Equipment .....	4
6. Procedure.....	4
6.1 Overview .....	4
6.2 Preparation of the filter paper .....	5
6.3 Collecting blood spots on filter paper.....	6
6.4 Storing the filter paper samples .....	6
7. References .....	7

## 1. Purpose

This procedure describes the preparation and preservation of filter paper dried blood spot samples for molecular genetic analysis.

## 2. Scope

This procedure is intended for use in clinical trials and molecular studies where blood spots from finger or heel sticks are taken for *Plasmodium* parasite DNA extraction. This procedure is applicable in well-equipped laboratories and requires some training.

## 3. Abbreviations

cm                    centimetre  
µL                    microlitre

## 4. Duties and Responsibilities

N/A

## 5. Materials and Equipment

- Whatman FTA® Elute cards (Whatman WB120410) or Whatman 3MM CHR chromatography paper (untreated)
- Scissors
- Ruler or straight edge
- Ink stamp, pencil or permanent ink pen
- Small sterile bags for storage of individual filter papers
- Large plastic storage bags
- Desiccant pouches
- Disposable latex or nitrile gloves
- Blood lancet
- Ethanol (95%)

## 6. Procedure

### 6.1 Overview

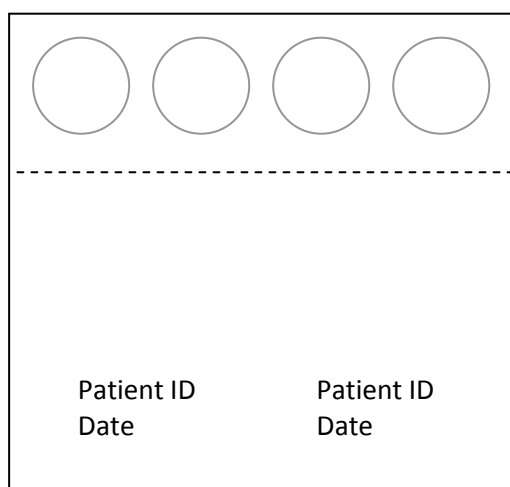
Blood samples collected on filter paper can be used for future molecular testing of extracted parasite and host DNA. Collection of samples on FTA® cards is preferable to untreated filter paper. FTA® cards are treated with agents that protect nucleic acids and prevent microbial growth, and can maintain stability of

DNA for years. If FTA® cards are unavailable, 3MM chromatography papers should be stored properly to improve DNA recovery and PCR success (see 6.4).

## 6.2 Preparation of the filter paper

**NOTE:** Gloves should be worn at all times when handling, labelling and cutting filter paper.

- 3MM chromatography paper should be pre-cut into individual squares, approximately 5 cm by 5 cm as shown:



**Figure 1.** Preparation and labeling of 3MM chromatography paper for four spots of ONE subject or patient's blood sample. Grey circles indicate approximate positions of each spot. Fold along dotted line. Image source: WWARN.



**Figure 2.** FTA® Micro Elute card for four spots of one patient's blood sample. Image source: <http://www.whatman.com/FTAElute.aspx>

- Stamp or label FTA® cards or 3MM chromatography paper to specify places to clearly write information such as subject or patient ID, initial, post-treatment day and today's date. Use pencil or permanent ink.

### **6.3 Collecting blood spots on filter paper**

- A filter paper sample should be collected at every time point requiring molecular analysis.
- The filter paper should be labelled with patient and sample information using pencil or permanent ink prior to performing the venipuncture or finger prick to obtain the blood sample. Ensure that writing is neat and firm so that the information can be easily read. The label should contain at least the date and patient ID and other pertinent information such as post-treatment day. Label twice to ensure clear identification (see Figure 1 for example).
- Fold the filter paper between the written information and the blood spotting location.
- If collecting blood using a finger prick (or heel stick in very young children), 2 to 3 drops of blood directly from the finger (or heel) should be gently touched to the center of each of up to 4 circles. As many drops as possible from a single prick should be collected.
- If using blood collected by venipuncture into a vacutainer tube, a micropipette or syringe and needle should be used to dispense approximately 50  $\mu$ L (one large drop) in the center of each of 4 spots.

### **6.4 Storing the filter paper samples**

- Blood-spotted filter papers should be left to air dry until completely dry. Do not apply heat to accelerate drying.
- Each dried filter paper sample should be stored in a separate small storage bag with a desiccant pouch.
- Bags with samples should be well sealed and kept in a dry place out of direct sunlight. Bags with samples collected on FTA<sup>®</sup> cards can be stored at room temperature. Bags with samples collected on untreated filter paper should be stored at 20° C if air conditioning is available, or at 4° C and with sufficient desiccant so that condensation does not occur.

## 7. References

MMV/World Health Organization, *Methods and techniques for clinical trials on antimalarial drug efficacy: Genotyping to identify parasite populations*, 2007.