

FIEBRE Standard Operating Procedure F.07a		
Title	Blood smear preparation and staining	
SOP Reference	Version	Date of effect
F.07a	1.1.1	10 Mar 2019

SOP Development

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Due date for next review	Reviewer name	Signature	Date reviewed
20 Apr 19			

Revision History

Version No.	Effective date	Reason for change

SOP User Confirmation

I acknowledge that I have read, understood and agree to follow this SOP

#	Name (print)	Signature	Date
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1 Title: BLOOD SMEAR PREPARATION AND STAINING

2 Purpose: To describe the procedures for preparing Giemsa-stained thick and thin blood smears for participants in the FIEBRE study.

3 Responsible staff: FIEBRE laboratory staff

4 Background & Rationale: For all patients in FIEBRE study, at time of enrolment, a thick and thin blood smear will be prepared, fixed and stained with Giemsa.

This will enable blood smear microscopy to be performed by expert microscopists (according to SOPs F-07b and F-07c) to look for presence or absence, density and species of *Plasmodium* alongside other non-malaria blood parasites and *Borrelia* spp.

5 Supplies and Materials

- Sample logbook [paper or ODK]
- Participant's blood sample in EDTA tube
- Sample labels with patient's QR code
- Gloves (single-use latex or vinyl)
- Clean glass slide
- Two (2) cover slips
- Glassware and racks for staining/drying
- Giemsa **3%** stain working solution (stable at room temperature for up to 8 hours)
 - Giemsa stock solution
 - Giemsa buffer solution (stable at room temperature for up to 7 days)
 - Liquid storage container (jerry can)
 - Measuring cylinders
 - Wash bottles
- Absolute methanol, in a container suitable for dipping a single glass slide
- DPX mountant
- Applicator sticks for DPX
- Paper towels

- Calibrated pipette and pipette tips or single-use plastic/Pasteur pipette
- Sharps bin
- Biohazard disposal system

6 Procedures:

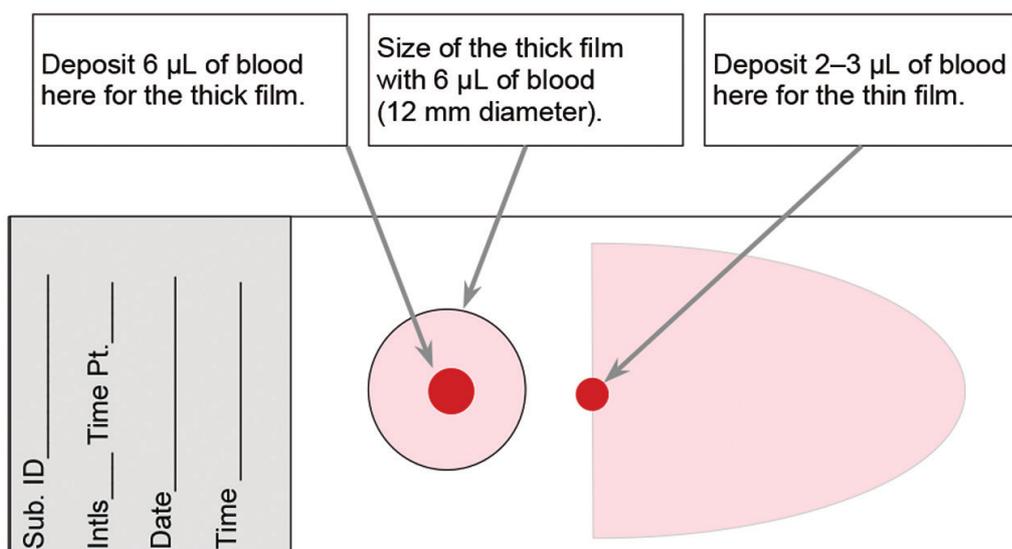
These are **site-specific**. The *preference* is that the thin and thick smears are prepared according to WHO methods manual i.e. the thin and thick smears on **one slide**. If this is too logistically or technically challenging at your site, it is acceptable to prepare thin and thick smears on separate slides.

Please note this SOP requires **3%** Giemsa stain to be used as a standard across all sites.

Ensure EDTA sample is inverted gently approximately 10 times or until mixed thoroughly before making slides. Or finger prick blood can be used placed directly onto clean microscope slide. Ideally make films within 24 hours of EDTA sample being taken.

6.1 Preparation of thick and thin smears for staining

6.1.1 Place 6 μL of blood for the thick film and 2–3 μL for the thin film as shown in the figure below



[Figure from WHO methods manual 'Microscopy for the detection,

identification and quantification of malaria parasites on stained thick and thin blood films in research settings' Copyright © World Health Organization on behalf of the Special Programme for Research and Training in Tropical Diseases 2015]

6.1.2 To make the thin film, use a spreader or another slide, at an angle of approximately 45 degrees, bring the spreading slide backwards into the drop of blood, allowing it to spread along the edge. Push the spreader forward in a steady movement to produce a film that has two straight edges with a feathered 'tail' that does not reach the end of the slide

6.1.3 Using the bevelled corner of the spreader slide spread the blood for the thick film until the entire circle of 12 mm diameter is covered evenly

6.1.4 Dry the films on a flat surface, protected from dust and insects. Slides must be completely dry before staining by drying on a slide warmer at 37–40 degrees for 1 hour or overnight in a dehumidified chamber at ambient temperature

6.1.5 Fix the thin film by dipping it in absolute methanol for a few seconds and then letting the slide air dry. Dry the thin film at an acute angle, with the film-side of the slide facing up and the thin film downwards. This protects the thick film from being fixed by methanol fumes and run-off. The thick film must not be fixed.

6.2 Staining thick and thin blood smears

6.2.1 Estimate the amount of 3% Giemsa stain working solution needed for the number of slides to be stained and prepare immediately before use

6.2.2 Fix each thin film, preferably using a Pasteur pipette or by dipping the thin film for 2 seconds into a small container or beaker containing methanol. Avoid contact between the thick film and methanol as the thick film must not be fixed (methanol and its vapours interfere with haemolysis of the thick film)

6.2.3 Place the blood film on tray/drying rack. Allow methanol-fixed thin smear to dry completely in air (around 2 minutes) by placing the slides on flat surface. Slide should never be allowed to dry in a vertical position with the thin film down, as this may result in fixation of thick film by vapour

6.2.4 Place slides in staining trough, making sure thick films are together at one end of the tray.

- 6.2.5 Pour stain gently into staining tray. Do not pour it directly onto the thick films as they may float off slides.
- 6.2.6 Set timer for 45-60 minutes (exposure time determined according to site environment)
- 6.2.7 Gently pour buffered water into the tray. To avoid disturbing thick films, pour water into thin film end.
- 6.2.8 Pour off the remaining stain and rinse with buffered water
- 6.2.9 Remove slides, one by one, and place film side down on drying rack to dry
- 6.2.10 When slides dry, mount with DPX mountant and cover slip for longevity of samples

6.3 Preparing thick and thin smears for staining on separate slides (please note this is only to be done where logistically or technically the smears cannot be prepared on one slide)

6.3.1 THICK FILM

- A. Place two separate 5ul (microlitre) drops of blood onto a clean microscope slide (leaving enough space between them for spreading)
- B. Spread each drop rapidly and evenly with the corner of another slide to form two circular areas measuring approximately 1.5-2cm in diameter
- C. Clearly label the slide at the frosted end with pencil
- D. Leave the film to dry at room temperature for 15 minutes
- E. Place the film into a 37°C incubator for 5 minutes
- F. The film is now ready to stain

6.3.2 THIN FILM

- A. Place a small drop of blood (between 2-5ul depending on viscosity of sample) at one end of a clean microscope slide
- B. Using a spreader or another slide, at an angle of approximately 45 degrees, bring the spreading slide backwards into the drop of blood, allowing it to spread along the edge
- C. Push the spreader forward in a steady movement to produce a film that has two straight edges with a feathered 'tail' that does not reach the end of the slide
- D. Clearly label the slide at frosted end with pencil

E. Allow the film to dry at room temperature

F. The film is now ready to stain

6.4 Staining thick and thin blood films when on separate slides

Use same staining protocol with 3% stain as in section 6.2

6.5 Storage of Giemsa-stained slides

Please note, for sites with expert microscopists, malaria microscopy will be performed on site (see SOP F-07b).

6.5.1 Giemsa-stained slides should be carefully stored in slide boxes at ambient temperature (not refrigerated)

6.5.2 A proportion of slides will be sent for external quality assurance as per SOP F-07c

7 Documentation: FIEBRE protocol (version 2.5, 31 Jul 2018) section 7.6, [WHO standard manuals for malaria microscopy and quality control in research settings and WHO MM-SOP-04 and MM-SOP-07A], SOPs F-07b and F-07c