

# **SOP**

## **Collection of finger prick blood, preparation and staining of thick and thin blood smears for Malaria diagnosis**

**Department of Parasitology  
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# Standard Operating Procedure (SOP) for Collection of finger prick blood, preparation and staining of thick and thin blood smears for Malaria diagnosis

## Issued By

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## 1. Purpose

The purpose of this SOP is to describe the standardized procedure for preparing thick and thin blood smears for diagnosing malaria (asymptomatic parasitemia, clinical malaria)

The careful examination of a well-prepared and well-stained blood smear currently remains the “gold standard” for malaria diagnosis.

## 2. Scope

This SOP applies to all academic staff, non-academic staff and students involved in parasitology practical sessions and diagnostic laboratory work related to direct microscopic visualization of the malaria parasite.

This SOP includes,

1. Collection of finger-prick blood
2. Preparation of Thick and Thin blood smears
3. Staining of the smears with Giemsa stain

## 3. Responsibilities

### 3.1 Academic Staff (Lecturer/Demonstrator)

- Supervise and guide students during collection of finger prick blood and slide preparation
- Ensure adherence to laboratory safety and procedural standards

### 3.2 Students

- Prepare slide according to the standard procedure
- Ensure proper labeling and handling of specimens
- Maintain cleanliness and avoid contamination

### 3.3 Technical officer

- Ensure availability of required materials and reagents
- Maintain quality assurance in laboratory procedures
- Prepare and standardize reagents and protocols

### 3.4 Laboratory assistant

- Clean and maintain laboratory equipment
- Arrange materials for practical sessions
- Dispose of waste properly

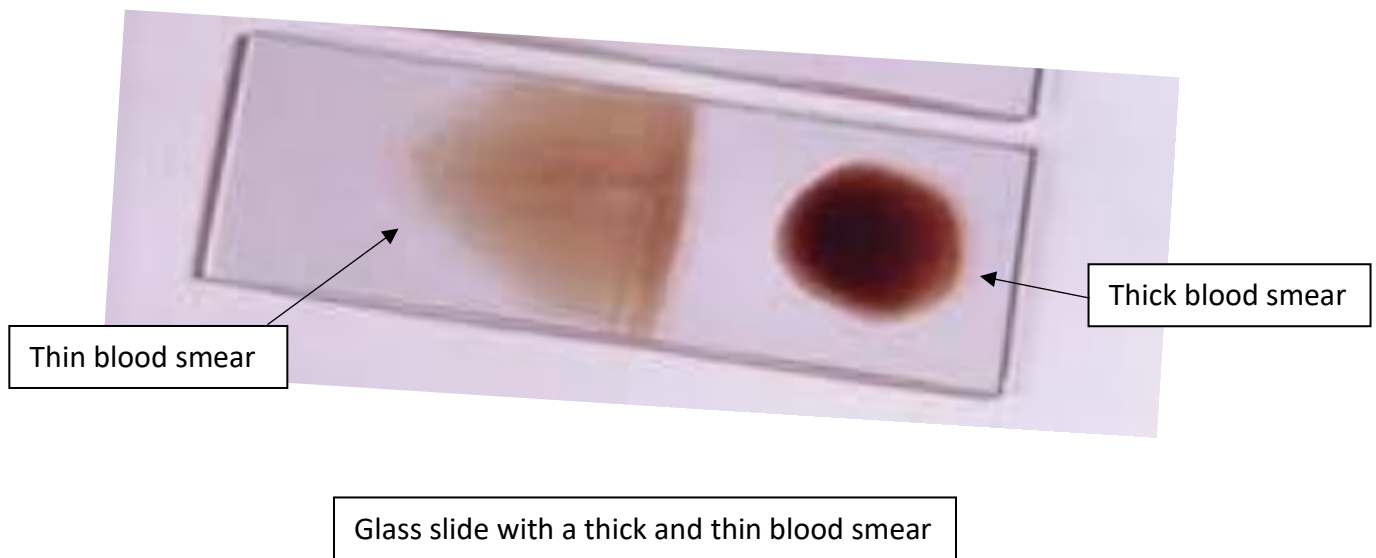
## 4. Importance of preparing thick and thin blood smears in malaria diagnosis

### 4.1 Thick blood smears –

- Consist of a thick layer of **lysed** red blood cells
- Thus, allows more efficient detection of parasites against a more transparent background even if the parasitemia is low
- However, do not permit an optimal review of parasite morphology
- So, it is generally used to make the diagnosis of malaria and calculate parasite density

### 4.2 Thin blood smears –

- Consist of thin layer of **intact/non lysed** red blood cells
- Here, blood spread in a layer, such that the thickness decreases progressively toward a monolayer.
- Thus, it allows to determine the different species and the stages of malaria parasites present



## 5. Safety precautions in handling a blood sample

The collection and handling of blood samples present a potential risk of infections. (especially Hepatitis, Acquired Immunodeficiency Syndrome (AIDS), Hepatitis)

This risk can be reduced by taking the following precautions:

- Wear protective gloves when handling or collecting blood
- Avoid contact of blood (including from unstained slide) with fingers or hands.
- Cover any cuts or abrasions on your hands with waterproof dressings.
- Take care not to prick yourself or others with any sharp instrument that has been in contact with blood.
- Do not reuse disposable lancets.
- Always wash your hands with soap and water after handling blood.
- If blood contacts skin, wipe immediately with alcohol-soaked cotton and wash with soap and water as soon as possible.
- Dispose of sharps (lancets, broken glass) in 'sharps containers' for incineration or autoclaving.
- Dispose of non-sharp blood-contaminated waste, in covered containers or autoclave bags for incineration or autoclaving.
- Collect blood in an area where there is proper lighting.

## 6. Materials and reagents need,

- Clean glass slide, 25 x 75 mm



- 70% ethanol or alcohol swabs (for disinfecting the finger)



- sterile lancets, one per patient (to prick the finger)



- dry cotton ball (to wipe the remaining blood from the finger)



- Latex gloves



- Sharps disposal container (to dispose sharps -lancets, broken glass)



- an infectious waste container (to dispose of biohazardous materials like blood-soaked cotton balls to prevent infection spread)



- a slide tray (to place the slide horizontally and allow air-dry)



- Wax pencil or permanent marker pen (to label the slide)



- Giemsa stain (10% or 3% solution)



- Absolute methanol, acetone free



- Pasteur pipette with a rubber teat (to fix the thin blood smear by carefully dropping methanol onto the thin blood)



- beakers for Giemsa stain and methanol



- Slide staining rack (to place the slides when staining)



- Timer (to set time for procedures)



## 7. Procedure

### 7.1 Labeling the slide

- Label the slide with a wax pencil or permanent marker pen by writing at one end of the slide.
- The label should contain the patient's name/reference number and date.



### 7.2 Wearing latex gloves, disinfecting the finger and stimulation of blood circulation

- Wearing latex gloves, select a finger of the nondominant hand. Do not use the thumb for either children or adults.
- Hold the patient's hand, palm facing upwards, and clean the selected finger with a piece of cotton soaked lightly in 70% ethanol or alcohol swab.
- Use firm strokes to remove dirt and oil from the ball of the finger and to stimulate blood circulation. Make sure the finger is warm by applying gentle massage if required. Let the alcohol dry from the finger.



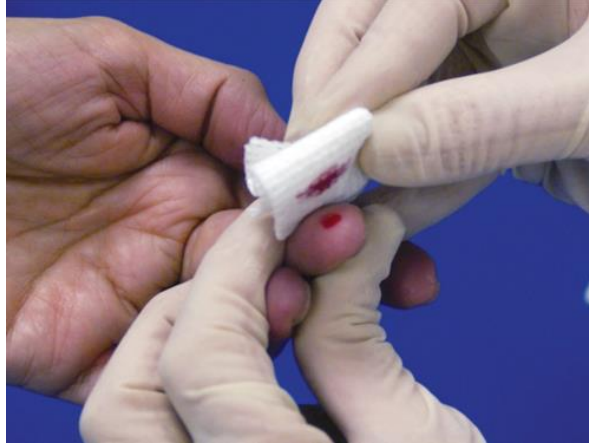
### 7.3 Prick the finger with a new, sterile lancet

- Using a new, sterile lancet puncture the center of the ball of the finger



#### 7.4 Express the first drop of blood and wipe it off with dry cotton

- Apply gentle pressure to the finger and express the first drop of blood.
- Wipe the first drop of blood off with dry cotton, making sure that no cotton strands remain on the finger that might stick to the blood.



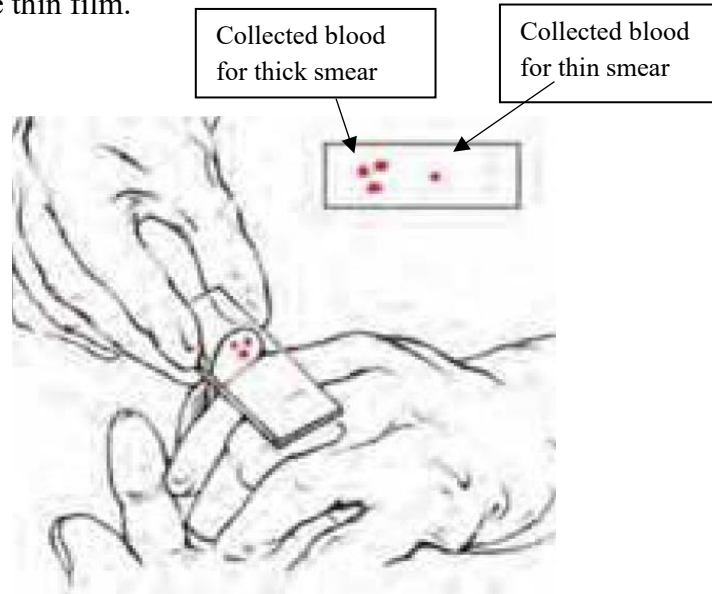
#### 7.5 Collection of blood for the thin smear

- Collect blood by applying gentle pressure to the finger and touching the slide to the blood
- Collect a single small drop of blood on the middle of the slide
- This is for preparation of the thin smear



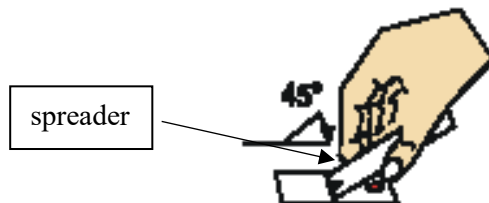
## 7.6 Collection of blood for the thick smear

- Apply further gentle pressure to express more blood, and collect two or three drops on the slide about 1 cm from the drop intended for the thin film.



## 7.7 Preparation of thin smear

- To prepare the thin film, use a second clean slide as a “spreader”.
- Place the edge of the clean spreader slide at an angle of  $45^\circ$  in front of the blood drop intended for the thin film.
- Slowly pull the spreader back until it touches the drop of blood and wait until the blood spreads along the entire width of the spreader.



- While holding the spreader slide at the same angle, push the spreader forward (away from the center) rapidly and smoothly, until the spreader leaves a “feathery” end for the thin film.

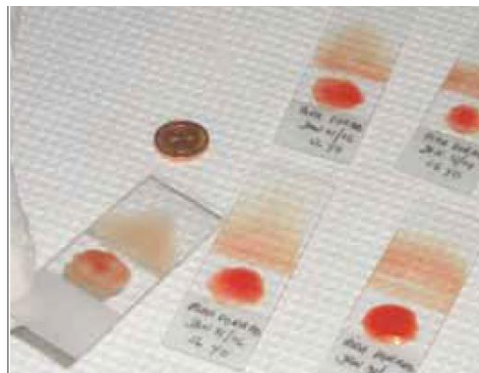


## 7.8 Preparation of thick smear

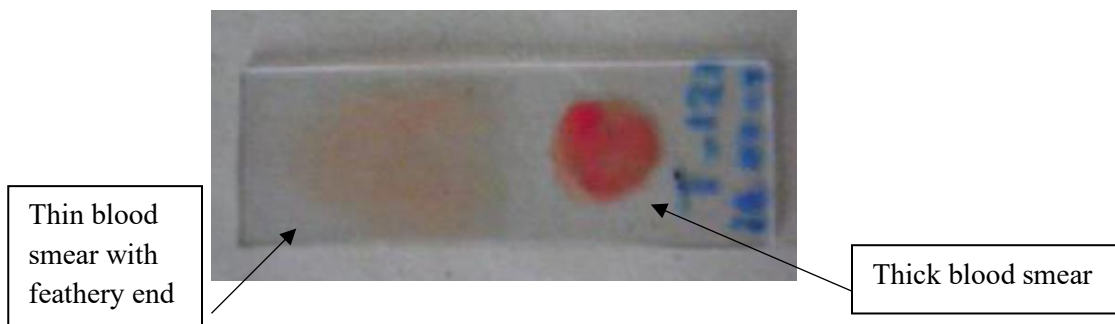
- Using the corner of the same spreader used for the thin film, make the thick film by gently spreading the three drops of blood together to form a circle of about 1 cm in diameter.
- Do not make the smear too thick as it will fall off the slide (thickness check: you should be able to read the newsprint through it)



- Allow the smears to dry thoroughly in a flat surface, before staining.  
(Note: Insufficiently dried or too thick smears can detach from the slide during staining.)



- The thin and thick blood smears for staining looks as given below.



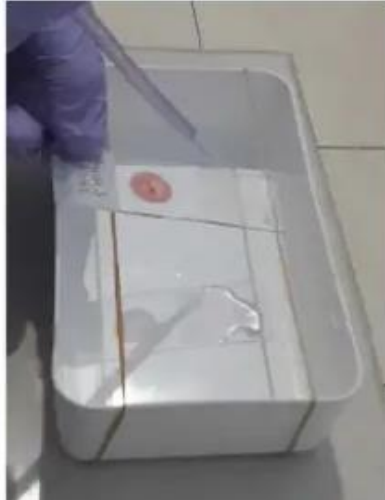
## 7.9 Staining of thick and thin blood smears

### ➤ Fixation

- **Only** thin blood smear must be fixed with methanol before staining with Giemsa stain.

(Note – Fixation with methanol before staining prevent the lysis of red blood cells and preserve the structure of red blood cells and malaria parasites inside those intact red blood cells, which is essential for species identification)

- Fix the thin film by carefully dropping methanol for 15-20 seconds onto the thin blood smear, Using a Pasteur pipette.



- Place the slide on a slide tray (flat surface) and let the thin blood smear to dry completely before staining, for 2 minutes.

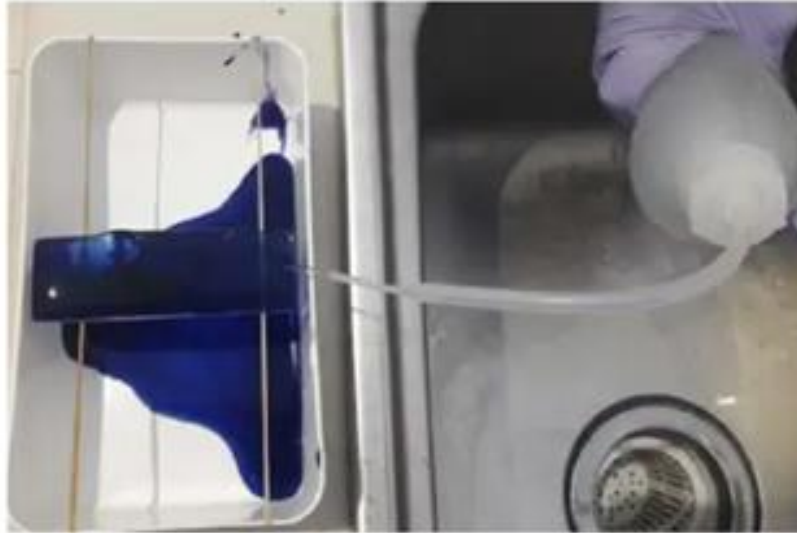
(Note: Never dry slide in a vertical position with the thin film down, as this may lead to fixing of the thick smear by methanol vapor)

### ➤ Staining with Giemsa stain

- Place the slide on a staining rack with blood smears facing up.
- Gently pour the stain onto the top of the slide until the blood films are covered with stain.



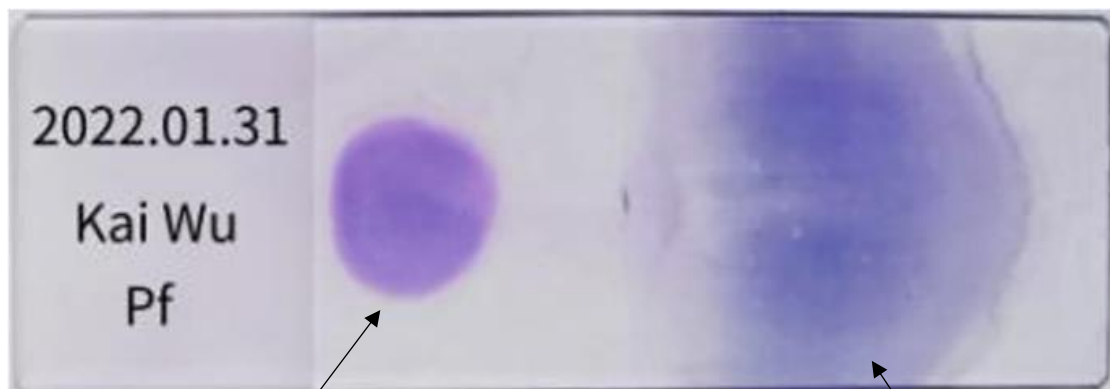
- Set the timer for the staining.
- Leave the stain on the slide for 45-60 minutes (if use 3% Giemsa solution) or 8-10 minutes (while using 105 Giemsa solution)
- Gently pour off the remaining stain and rinse with clean water.



- When the stain has been washed away, allow the slide to air-dry on slide tray.

(Note -Never fix thick blood smear with methanol. The thick blood smear should be stained with Giemsa stain immediately to prevent auto-fixation if exposed to extreme heat. Reason – Immediate staining is done to prevent the auto-fixation of the smear and facilitate the lysis of red blood cells, allow intact stained parasites to come out from the red blood cells and clearly visible against a clear background)

- The stained thin and thick blood smears for examination under microscopy looks as given below.



Stained thick blood smear

Stained thin blood smear

## 8 References

- WHO guidelines in preparation of thick and thin blood films for malaria diagnosis
- Practical handbook issued by Faculty of Medicine, University of Colombo

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