PREPARATION OF BLOOD FILMS FOR MALARIA DETECTION

Materials for Preparation of Malaria Smears:
- Clean and wrapped slides
- Sterile lancets
- 70% ethanol and water
- Absorbent cotton wool
- Surgical gloves
- Lint-free cotton cloth
- Soft lead pencil
- Ball-point pen

Making the Malaria Smear:
Making the Malaria Smear Holding the patient’s left hand, palm upwards, select the third finger from the thumb. The big toe can be used with infants. The thumb should never be used for adults or children. Clean the finger with a piece of cotton wool lightly soaked in 70% ethanol, using firm strokes to remove grease and dirt from the ball of the finger. Dry the finger with a clean cotton cloth, using firm strokes to stimulate blood circulation.

Perform Finger Stick:
Puncture the ball of the finger with a sterile lancet, using a quick rolling action. Apply gentle pressure to the finger to express 1st drop of blood and wipe it away with a dry piece of cotton wool. Be sure cotton strands do not remain on the finger and mix with blood. Perform Finger Stick. (Make sure to properly dispose used lancets in a puncture proof container)

Collecting Blood for Thick Prep:
Working quickly, handling slides by the edges, collect the blood using the following procedure Apply further pressure to express more blood and collect two or three larger drops, about this size ● on the slide, about 1 cm. Wipe finger with cotton. Allow the thick film to dry with the slide in a flat, level position, protected from flies, dust and extreme heat.
Collecting Blood Sample for Thin Prep:
Collecting Blood Sample for Thin Prep Working quickly, handling slides by the edges, collect the blood using the following procedure. Apply further pressure to express blood and collect two or three larger drops, about this size (circle), on the slide, about 1 cm. Proceed with making (thin) smear as prepared for blood cell identification and enumeration. Wipe finger with cotton.

Making a Thin Film:
Using a second clean slide as “spreader” and, with the slide with the blood drops resting on a flat, firm surface, touch the small drop with the spreader and allow the blood to run along its edge. Firmly push the spreader along the slide, keeping the spreader at an angle of 45. Make sure that the spreader is in even contact with the surface of the slide all the time the blood is being spread.

Making a Thick Film:
Making a Thick Film Using the corner of the spreader, quickly join the drops of blood and spread them to make an even, thick film. The blood should not be excessively stirred but can be spread in circular or rectangular form with 3 to 6 movements. The circular thick films should be about 1 cm (1/3 inch) in diameter.

Labeling the Slide:
Label the frosted edge using a soft lead pencil with the Serial Number and date of collection. Do not use a ball-point pen for labeling the slide.
COMMON FAULTS IN MAKING BLOOD FILMS

A number of faults are common in making blood films. These can affect the labeling, the staining or the examination, and sometimes more than one of these.

Badly positioned blood films

Care should be taken that the blood films are correctly sited on the slide. If they are not, it may be difficult to examine the thick film. Also, portions of the films may even be rubbed off during the staining or drying process.

Too much blood

After staining films made with too much blood, the background to the thick film will be too blue. There will be too many white blood cells per thick film field, and these could obscure or cover up any malaria parasites that are present. If the thin film is too thick, red blood cells will be on top of one another and it will be impossible to examine them properly after fixation.

Too little blood

If too little blood is used to make the films, there will not be enough white cells in the thick film field and you will not examine enough blood in the standard examination. The thin film may be too small for use as a label.

Blood films spread on a greasy slide

The blood films will spread unevenly on a greasy slide, which makes examination very difficult. Some of the thick film will probably come off the slide during the staining process.
Edge of spreader slide chipped

When the edge of the spreader slide is chipped, the thin film spreads unevenly, is streaky and has many "tails". The spreading of the thick film may also be affected.

Thin film too big, thick film in the wrong place

If the thin film is too large, the thick film will be out of place and may be so near the edge of the slide that it cannot be seen through the microscope. During staining or drying, portions of the thick film will probably be scraped off by the edges of the staining trough or drying rack. It may be very difficult, or impossible, to position the thick film on the microscope stage so that it can be examined.

Other common faults

Other faults that occur commonly in the preparation of blood films include the following:

• Flies, cockroaches or ants eat the dry blood and damage the films.

• Blood films are made on badly scratched slides.

• The thick film is allowed to dry unevenly.

• Auto fixation of the thick film occurs with the passage of time or through exposure to heat, and staining then becomes difficult or unsatisfactory.

• Slides are wrapped together before all the thick films are properly dried, and the slides stick to one another.
STAINING BLOOD FILMS

Materials

Giemsa stain in a 25-ml bottle
Methanol¹
Absorbent cotton wool
Test tubes, capacity 5 ml
Distilled/deionized water, buffered to pH 7.2
Pasteur pipette, with rubber teat
Curved plastic staining tray or plate
Slide-drying rack
Timing clock
Small electric hair-drier or spirit lamp

Method

Thick blood films must be thoroughly dry before they are stained. They can be dried more quickly with warm air blown from a small hair-drier or by exposing them to the heat from a spirit lamp. However, great care must be taken to avoid making slides hot to the touch, otherwise films will be heat-fixed and will not stain properly.

Step 1 Fix the thin film by dabbing it with a pledget of cotton wool dampened with methanol or by dipping it in a container of methanol for a few seconds. Avoid methanol, or its fumes, coming into contact with the thick film, otherwise fixation may take place and will prevent proper staining.

Step 2 Use a test-tube or small container to hold the prepared stain. Make up a 10% Giemsa solution with distilled/deionized water buffered to pH 7.2. If only one slide is to be stained, you will require about 3 ml of prepared stain. Allow 3 drops of stock Giemsa solution (from the Pasteur pipette) to each millilitre of buffered water to give a 10% solution.
Step 3 Gently pour the stain on to the slides (or use a pipette to drop the stain on to the slide).

Step 4 Stain the film for 5 to 8 minutes. Experience will indicate the correct time for each slide (or batch of slides).

Step 5 Gently flush the stain off the slide by adding drops of clean water. Never pour the stain off the slides, otherwise the surface scum will stick to the film and spoil it for microscopic examination.

Step 6 Place the slide in the drying rack, film side downwards, to drain and dry. Make sure that the thick film does not touch the edge of the rack.

Credit Adapted from: WHO – Geneva Bench Aids for Diagnosis of Malaria Infection, 2nd Edition
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