## Transcript: How to Perform a Proper Thick and Thin Smear for Malaria Diagnosis

In order to perform a proper thick and thin blood smear you need the following material:

- Gloves as universal precaution
- Dry and clean pieces of gauze
- A spreader
- Clean and clear Glass Slides of 25 X 76 mm approximately
- Small cotton balls soaked in alcohol
- Sterile blood lancets
- A bin to discard used blood lancets
- A slide folder to protect slides from dust & flies

As a spreader you need a slide with CLEANED FROSTED AND POLISHED edges: when you pass your finger on the edge it should feel very smooth. If you feel any asperities or surface roughness you should replace the spreader.

Health workers should be comfortably seated, with enough bench space and proper lighting

For pricking the finger choose the middle finger or the ring finger. Always prick on either side of the fingertip, NOT IN THE MIDDLE. This is because the middle of the fingertip has more nerve endings than the sides. Therefore pricking on the side is less painful for the patient.

The patient should be seated comfortably.

Then record a request form and a slide (here it is done with a sticker but it can be done on the thin smear with a pencil). RECORD SHOULD HAVE AT LEAST A CODE OR A NAME AND THE DATE.

Check the slide is clean free of dust or finger prints. If not, clean it with a dry piece of gauze. Note here that the slide can be manipulated as long as you keep your fingers on the edges of the slide.

Massage fingertip to increase blood flow.

Clean the fingertip thoroughly with a cotton ball soaked in alcohol. This step needs to be done properly in order to avoid contamination of the blood taken or infection for the patient.

Open the protective wrapper of the sterile blood lancet from the back side. If you notice the protective wrapper has already been teared off or damaged, discard the blood lancet as it is no longer sterile and use a new one.

Pull the lancet out of the protective wrapper and prick the side of the fingertip with a quick hand rotation.

Discard the used blood lancet in a proper bin

Press slightly the finger. The blood should come out easily. If not, restart by cleaning and pricking another finger.

WITH A DRY & CLEAN PIECE OF GAUZE WIPE OUT THE REMAINING ALCOHOL & THE FIRST DROP OF BLOOD AS IT MAY CONTAIN SKIN DEBRIS.

Press the finger to have a blood drop out. Come with the slide to make contact with the top of the blood drop BUT NOT WITH THE FINGER. This is crucial if you want to take a standard blood quantity and have thick blood smears with standard thickness. Repeat this in order to obtain 2 to 3 drops of blood for the thick smear. If the drops are small take 3 if they are a bit bigger take 2. The volume of blood for the thick smear should be about 6 to 10 microliters.

For the thin smear take one small drop (2 to 3  $\mu$ l) about 1 cm away from the thick smear and in the middle of the slide.

Give back the cotton ball to the patient, telling him to press on the puncture site for a minute.

Hold the slide between your thumb and both your middle and index finger. With the other hand take the spreader and put it in contact with the slide in front of the thin smear drop. Move the spreader backward to make contact with the blood drop. Wait for the blood to run along the spreader's edge. You can help that process by opening and closing the angle of the spreader in quick succession.

Keeping an angle of approximately 45° move the spreader forward with a regular move. There is no need to apply too much pressure on the spreader but it should stay in even contact with the slide surface when the blood is being spread. The forward move should be regular, not too fast and completed until the spreader reaches your thumb.

Then put the slide on the bench. With a corner of the spreader join the 2 or 3 drops for the thick blood film with few circular movements, in order to obtain a circle of about 12mm in diameter.

Place the slide in a slide folder to protect it from dust and flies. Leave it there until blood has completely dried. THE FOLDER SHOULD BE ON A FLAT AND LEVEL POSITION. IF NOT THE BLOOD FOR THE THICK BLOOD FIM WILL RUN ON ONE SIDE: SO YOU END UP WITH HALF OF YOUR THICK SMEAR TOO THICK AND THE OTHER HALF TOO THIN.

Then clean thoroughly the edge and corner of the spreader with a cotton ball soaked in alcohol and then a clean piece of gauze. The spreader must be cleaned before you proceed to the next patient in order to avoid contamination or parasites transfer from one slide to another.

Here it is just to show you that when the drops are bigger only 2 will be enough for the thick smear. On a standard thick smear before it dries, you should be able to see newsprint through the thick smear but barely able to read it. After staining, a standard thick smear on a patient with a normal WBC count should have approximately 10 to 15 WBC per HPF.

To perform a good thin smear, your spreader should go forward in a regular move and not too fast. One of the common mistake is that a too big drop of blood is applied on the slide and then, you need to spread the blood fast in order to keep the thin smear within the further edge of the slide.

REMEMBER: a small drop of blood (2 to 3  $\mu$ l) is enough for the thin smear.

On a well performed thin smear the tail of thin smear should dry very quickly and looking through, you should be able to see rainbow like colors in that area.

Here I complain with my staff who forgot to clean the spreader after smearing.

- Stephane Proux, Malaria Diagnosis Lab Supervisor at Shoklo Malaria Research Unit (SMRU)