1. Scope and application

- This procedure describes the preparation of thin blood films
- Thin blood films are used for differential leucocyte count, the detection of abnormal erythrocytes, thrombocyte count, the detection of certain parasites (e.g. for the species identification of *Plasmodium* parasites, the causal agent of malaria, *Trypanosoma* sp., blood microfilaria,…) and spirochetes (*Borrelia* spp.)

2. Responsibilities

<table>
<thead>
<tr>
<th>Function</th>
<th>Activities</th>
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<tbody>
<tr>
<td>Lab technician</td>
<td>• Collects the blood</td>
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<td>• Follows this procedure for preparation and staining of thin</td>
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<td>blood films</td>
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<td></td>
<td>• Is responsible for the correct storage of the reagents</td>
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<td>• Is responsible for the correct maintenance of the microscope</td>
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<td>• Informs the lab manager if there is a problem with the</td>
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<td>microscope</td>
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<td>• Participates in EQA programs when available</td>
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<td>QA manager</td>
<td>• Supervises the correct storage of the reagents</td>
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<td>• Supervises the maintenance schedule for the microscope</td>
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<td></td>
<td>• Enrolls the lab in an EQA program for malaria</td>
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<td>Lab manager</td>
<td>• Supervises the QA manager</td>
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<td>• Contacts the technical engineer if there is a problem with the</td>
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<tr>
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<td>microscope</td>
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3. References

- WHO:
  - Basic malaria microscopy, part 1 Learner’s guide, second edition, 2010
    [http://www.wpro.who.int/nr/rdonlyres/cfd9efcc-a0f8-425e-85d7-d65c1b050934/0/malaria_malaria_microscopy_learners_guide2010.pdf](http://www.wpro.who.int/nr/rdonlyres/cfd9efcc-a0f8-425e-85d7-d65c1b050934/0/malaria_malaria_microscopy_learners_guide2010.pdf)
  - Malaria microscopy quality assurance manual, version 1, 2009
- CDC bench aids:
  [http://www.dpd.cdc.gov/dpx/HTML/PDF_Files/Malaria_procedures_benchaid.pdf](http://www.dpd.cdc.gov/dpx/HTML/PDF_Files/Malaria_procedures_benchaid.pdf)

4. Procedures

4.1 Basic principle

The thin blood films are prepared from whole blood with clean, dry and wrapped microscope slides (cfr. WHO 2010, Basic malaria microscopy, part 1. Learner’s guide, second edition). After staining with Giemsa, the slide can be examined under the microscope.
Thin blood films have a low sensitivity for detection of malaria parasites. Therefore they are used in conjunction with thick blood films, in order to identify the malaria species that was detected by microscopic examination of the thick blood film.

4.2 Safety

- All blood samples are potentially infectious. Follow standard safety precautions.
- Giemsa stain is flammable. Operate this stain far from an open source of fire.

4.3 Specimen

- Special conditions for patient preparation: none
- Type of sample: capillary (finger prick) or anti-coagulated venous blood (EDTA)
- Volume of sample: 2 µl of whole blood
- Sterility: not needed
- Time between collection and measurement:
  - capillary: immediately
  - venous: within 1 hour after collection
- Rejection criteria:
  - hemolyzed or clotted samples
  - blood collected in sodium citrate or in tubes containing a gel separator
  - samples with bacterial or fungal infection
  - blood samples not or incorrectly labeled

4.4 Materials

- Clean and degreased microscopic slides with frosted ends
- Disposable gloves
- Capillary blood collection set (tissue or gauze, alcohol swabs, sterile lancets)
- Automatic pipette
- Yellow pipette tips
- Pasteur pipette
- Clean water (tap water)
- Cylinder of 10 and 20 ml
- Beaker of 200 ml
- Bottle of 1L
- Giemsa mother solution (Merck 1.09204.0500)
- Buffer tablets pH 7.2 (Merck 1.09468.0100)
- Staining bath or staining rack
- Drying rack
- Timer
- Methanol
- Ethanol
- Microscope, objective 100x
- Immersion oil

4.5 Preparation of the thin blood film

- Use clean utensils, glassware and slides for each blood sample
- Clean the slides with ethanol using a piece of soft cloth
- Put on disposable gloves
- Write down the patient number on the frosted part of the slide
- For venous whole blood:
  - Mix the EDTA blood sample thoroughly
  - Place 2 µl of blood at one side of the slide
- For capillary blood:
  - Perform a finger prick (cfr. SOP finger prick)
  - Touching the drop of blood lightly with one end of the slide
  - This way, collect a drop of blood about 4mm diameter
• Use another clean slide as a spreader.
• Place the edge of the spreader just in front of the drop of blood.
• Draw the spreader back, until it touches the drop of blood with the edge of the slide.
• The blood will spread along the edge.
• Push the spreader firmly along the slide, at an angle of 45°, in a smooth movement.
• The complete drop of blood is spread into a thin, homogeneous layer.
• Shake the slide immediately afterwards between two fingers to dry it instantly.

Figure 1: Practical Notes “Human Parasitology in Tropical Settings”, Postgraduate in Tropical Medicine and International Health, ITM, 2009, p. 106-108.

• The thin blood film should be thick enough at the starting point (1), becoming less thick in the middle (2), and have a “flame”-like shape at the end (3).

Figure 2: Practical Notes “Human Parasitology in Tropical Settings”, Postgraduate in Tropical Medicine and International Health, ITM, 2009, p. 106-108.

4.6 Preparation of buffered water (pH 7.2)

• Dissolve 1 tablet of pH-buffer in 1L of tap water.
• Verify the pH of the buffered water (with the pH meter or pH indicator strips). The pH should be between 7.0 – 7.4.
• Write “Buffer water pH 7.2”, the date of preparation and the expiry date on the bottle.
• The buffer water is stable for 1 month at room temperature.

4.7 Preparation of Giemsa solution 3.5%

• Prepare a fresh Giemsa solution 3.5% just before you start the staining.
• Each slide needs approximately 3 ml of stain to cover it.
• Prepare the volume of Giemsa solution needed for the amount of slides you want to stain
• For 1 slide:
  o Pour 4 ml buffer water in a cylinder
  o Add 5 drops of concentrated Giemsa solution
  o Mix gently
  o The solution is stable for 1 hour at room temperature

4.8 Staining of the slides

• Fix the thin film by dipping it briefly into methanol or poor some drops of methanol on the thin film.
• When the methanol is evaporated, place the slides in a staining trough (back to back, 20 slides/trough) or on a staining rack (depending on how much slides you have to stain)
  Do NOT let the slides touch each other.
• Staining in staining through:
  o Fill the staining trough completely with the freshly prepared Giemsa working solution 3.5%
  o Do NOT poor the stain directly onto the thick films
  o Let the slides stain for 20 minutes
  o After 20 minutes: gently poor clean water into the trough. Remove the dye and carefully rinse the slides one by one in a beaker with clean water
• Staining on staining rack:
  o Cover each slide completely with freshly prepared Giemsa working solution 3.5% using a Pasteur pipette
  o Let the slides stain for 20 minutes
  o Drain the slides
  o Rinse each slide carefully in a beaker with clean water
• Let the slides dry in a drying rack
• Examine the slides under a microscope, objective 100x, with immersion oil

Examples of Giemsa stained thin blood films, used for malaria species identification, by microscopy, 100x:

P. falciparum trophozoite  P. malariae trophozoites  P. falciparum schizont  P. falciparum gametocyte  P. vivax schizont

Figures 3 - 7: WHO, CD-rom: The microscopic diagnosis of malaria

4.9 Quality control

Verify the quality of the thin film before drying and fixing.
Examples of thin films that are not correctly prepared:

- The thin film is too long
- Too much blood
- A spreader with rough edges
- The slide is greasy
- Not enough blood

4.10 Storage of the slides

- When dry, verify the blood film quality and place the microscopic slides in a slide box
- Mark the index of the box with the patient number and species

5. Records and archives

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