World Health Organization

GIEMSA STAINING OF MALARIA BLOOD FILMS

MALARIA MICROSCOPY STANDARD OPERATING PROCEDURE – MM-SOP-07A

1. PURPOSE AND SCOPE

To describe the procedure for properly staining malaria blood films with Giemsa stain.

This procedure is to be modified only with the approval of the national coordinator for quality assurance of malaria microscopy. All procedures specified herein are mandatory for all malaria microscopists working in national reference laboratories, in hospital laboratories or in basic health laboratories in health facilities performing malaria microscopy.

2. BACKGROUND

A properly stained blood film is critical for malaria diagnosis, especially for precise identification of malaria species. Use of Giemsa stain is the recommended and most reliable procedure for staining thick and thin blood films. Giemsa solution is composed of eosin and methylene blue (azure). The eosin component stains the parasite nucleus red, while the methylene blue component stains the cytoplasm blue. The thin film is fixed with methanol. De-haemoglobinization of the thick film and staining take place at the same time. The ideal pH for demonstrating stippling of the parasites to allow proper species identification is 7.2.

Methods of staining

The two methods for staining with Giemsa stain are the rapid (10% stain working solution) and the slow (3% stain working solution) methods.

The rapid (10% stain working solution) method

This is the commonest method for staining 1–15 slides at a time. It is used in outpatient clinics and busy laboratories where a quick diagnosis is essential for patient care. The method is efficient but requires more stain. The need for speed justifies the additional cost.

The slow (3% stain working solution) method

The slow method is used for staining larger numbers of slides (≥ 20). It is ideal for staining blood films collected during cross-sectional or epidemiological surveys and field research and for preparing batches of slides for teaching. It is less appropriate when a quick result is needed. The slow method is less expensive than the rapid method because it requires much less stain (3% rather than 10% stain solution).

3. SUPPLIES AND MATERIALS

For the rapid (10% stain working solution) method

- Giemsa stain (10% solution) (See MM-SOP-04 for method of preparation);
- a small container or beaker for Giemsa working stain;
- absolute methanol, acetone-free;
- a Pasteur pipette with a rubber teat;
- a small container or beaker for methanol;
- a curved plastic staining tray, plate or staining rack;
- a timer;
- a slide-drying rack;
- a small electric hair-dryer;

- protective latex gloves, powder-free, disposable and
- Distilled or deionized water buffered to pH 7.2.

For the slow (3% stain working solution) method

- Giemsa stain (3% solution) (See MM-SOP-04 for the method of preparation);
- a small container for Giemsa working stain;
- absolute methanol, acetone-free;
- a Pasteur pipette with a rubber teat;
- a small container or beaker for methanol;
- staining troughs that can hold 20 slides placed back to back;
- a timer;
- a slide-drying rack;
- protective latex gloves, powder-free, disposable, and
- distilled or deionized water buffered to pH 7.2.

4. SAFETY PRECAUTIONS

1. Methanol (methyl alcohol) is inflammable and highly toxic if inhaled or swallowed; it can cause blindness and even death if swallowed in any quantity. Avoid contact and inhalation. When it is not in use, it should be stored in a locked cupboard.
2. Universal precautions – including use of relevant personal protective equipment such as gloves, safety glasses and a laboratory coat or gown – must be practised. See MM-SOP-11: General safety procedures in the malaria microscopy laboratory.

5. PROCEDURE

FLOW CHART	DESCRIPTION OF ACTIVITY
5.1 For the rapid (10%) method	**5.1 For the rapid (10%) method**
1. Prepare 10% Giemsa working solution (MM-SOP-04), and place it in a small container.	1. Estimate the amount of 10% Giemsa working solution required for the number of slides to be stained. Each slide requires approximately 3 mL of stain to cover it. Prepare the stain immediately before use according to MM-SOP-04: Preparation of Giemsa working solution.
2. Using a Pasteur pipette, fix the thin film by carefully dropping methanol onto the thin film only.	2. To fix the thin film, preferably use a Pasteur pipette or dip the thin film for 2 s into a small container or beaker containing methanol. Avoid contact between the thick film and methanol, as methanol and its vapours will quickly fix the thick film and interfere with haemolysis of the thick film.
3. Let the blood film dry in air on a drying rack or tray.	3. Place the slides on a tray or drying rack. Allow the methanol-fixed thin smear to dry completely in air (approximately 2 min) by placing the slides on a flat surface. Never let the slide dry in a vertical position with the thin film down, as this may lead to fixing of the thick film by methanol vapour.
4. Place slides for staining blood films face down on a curved staining tray or face up on a staining rack.	4. Place slides for staining blood films face down if using a curved staining tray or facing up if using a staining rack.
5. Pour stain slowly on or under the slide until the blood films are covered.	5. Pour the stain gently between the slide and the staining tray if staining face down, until each slide is covered with stain, or gently pour the stain onto the top of slides lying face upwards on a staining rack.
6. Set the timer to 8–10 minutes for the staining.	6. Set the timer to 8–10 min (the exposure time should be determined previously by testing the batch of stock staining solution used), and allow the blood films to stain. Experience with the stain you are using will help indicate the time required for good staining. See MM-SOP 3c: Quality control of Giemsa stock solution and buffered water.
7. Gently flush all the stain from the slides by dropping clean water over it.	7. At the end of the staining time, remove each slide individually. Gently flush the stain from the slide by adding drops of buffered water until all the stain has been washed away. Do not pour the stain directly off the slides, as the metallic green surface scum will stick to the film, spoiling it for microscopy.
8. Allow the slides to air-dry.	8. When the stain has been washed away, place the slide in the drying rack film side downwards, or in a vertical position with the thick film down to drain and dry. Ensure that thick films are not scraped against the edge of the rack.
9. Discard the remaining 10% Giemsa solution.	9. Discard the remaining 10% Giemsa solution.

FLOW CHART	DESCRIPTION OF ACTIVITY
5.2 For the slow (3%) method	**5.2 For the slow (3%) method**
1. Prepare a 3% Giemsa working solution (MM-SOP-04), and place it in a small container.	1. Estimate the amount of 3% Giemsa stain working solution needed for the number of slides to be stained. Prepare the stain immediately before use according to MM-SOP-04: Preparation of Giemsa working solution.
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2. Fix only the thin film with methanol. Avoid contact between the thick film and methanol to avoid accidental fixation.	2. Fix each thin film, preferably using a Pasteur pipette or by dipping the thin film for 2 s into a small container or beaker containing methanol. Avoid contact between the thick film and methanol, as methanol and its vapours quickly fix thick films and interfere with the haemolysis of the thick film.
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3. Allow the blood film to dry in air on a drying rack or tray.	3. Place the blood film on a tray or drying rack. Allow the methanol-fixed thin smear to dry completely in air (approximately 2 min) by placing the slides on a flat surface. Never let the slide dry in a vertical position with the thin film down, as this may result in fixation of the thick film by methanol vapour.
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4. Place the slides back-to-back in a staining tray.	4. Place the slides back-to-back in a staining trough, making sure that the thick films are together at one end of the tray.
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5. Pour stain slowly on the slides. Do not pour it directly onto the thick films.	5. Pour the stain gently into the staining tray. Do not pour it directly onto the thick films, as they may float off the slides.
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6. Set the timer to 45-60 minutes and stain the blood film.	6. Set the timer for 45–60 min (the exposure time should be determined previously by testing the batch of stock staining solution), and stain the blood films. Experience with the stain you are using will help indicate the time required for good staining. See MM-SOP 3c: Quality control of Giemsa stock solution.
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7. Gently pour clean water into the tray to float off the iridescent scum.	7. Gently pour buffered water into the tray to float off the iridescent "scum". To avoid disturbing the thick films, pour the water into the thin film end. A less satisfactory way of flushing slides is to immerse the whole tray in a basin filled with clean water, making sure to avoid the iridescent scum when removing the tray from the basin.
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8. Gently pour off the remaining stain, and rinse with clean water.	8. Gently pour off the remaining stain, and rinse with buffered water.
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9. Carefully remove the slides, and allow them to dry.	9. Carefully remove the slides, one by one, and place them film side down in the drying rack to dry. Make sure that the thick films do not touch the edge of the rack.
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10. Discard the remaining 3% Giemsa solution.	10. Discard the remaining 3% Giemsa solution.

6. PROCEDURE NOTES

Drying the thick blood film

Thick blood films must be completely dry before being stained. They can be dried quickly with warm air from a small hair-dryer. Avoid overheating slides, as they can "heat fix" and thus stain poorly.

Use of buffered water for rinsing slides

The pH of the water used for rinsing is important, as acidic water may decolorize the films. It is therefore recommended that slides be rinsed with the same buffered water that is used for staining and therefore has a pH of 7.2 .

Care of glassware and measuring equipment

Measuring cylinders, pipettes, staining troughs and beakers must be clean and dry before use. Staining blood films with dirty utensils gives unsatisfactory results.

The equipment used for Giemsa staining should be rinsed immediately after use in clean water to remove as much of the stain as possible. It should then be soaked for a while in a detergent solution before washing. Utensils can be washed with a mild detergent, provided they are rinsed thoroughly in clean water before drying. Any detergent that is left on glass- and plastic-ware can alter the pH of the water and the stain, resulting in poor staining when the equipment is next used.

Caution

During staining with Giemsa stain (3% or 10% stain working solution), the surface becomes covered with a metallic green scum. Avoid getting it onto blood films during rinsing, as it can impair examination.

Blood smears should be stained as soon as possible after they are prepared. Storage of unstained slides for a few days in hot, humid conditions before staining will result in auto-fixation, and the thick film will be rendered useless for microscopy.

Method for staining individual slides

1. Place the slides individually on the staining rack, making sure that they are not touching each other.
2. Pour the stain gently onto the slides until they are totally covered. Each slide will require approximately 3 mL of stain. Avoid pouring the stain directly onto thick films.
3. Leave the stain on the slides for 45-60 min with 3% Giemsa solution and 10–15 min with 10% Giemsa solution. Internal quality control of your stain will indicate the optimum staining time.
4. Flood the slides gently with buffered water to float off the iridescent "scum" on the surface of the stain. Water buffered to 7.2 pH should be poured onto the slides from the thin film end to avoid undue disturbance and washing-off of the thick films.
5. Remove the slides one by one and place them, thick film downwards, in a drying rack to drain and dry, making sure that the thick film does not touch the edge of the rack.

7. RELATED SOPs

MM-SOP-3c: Quality control of Giemsa stock solution and buffered water

MM-SOP-04: Preparation of Giemsa working solution

8. REFERENCES

Basic Malaria Microscopy. Part I. Learner's Guide, Second Edition. WHO. 2010

Storey J. Standard operating procedures for Giemsa malaria microscopy. 2012. Unpublished.

9. DOCUMENT HISTORY

Date (mmm/yyyy)	Version	Comments	Responsible person (First name, last name)
Jan 2016	1	Reviewed and finalized by experts, edited and formatted	Glenda Gonzales, Technical Officer, WPRO