

# PREPARATION OF GIEMSA WORKING SOLUTION

## MALARIA MICROSCOPY STANDARD OPERATING PROCEDURE – MM-SOP-04

### 1. PURPOSE AND SCOPE

To describe the procedure for preparing a working solution of Giemsa stain from the stock solution for routine staining of malaria blood films

This procedure is to be modified only with the approval of the national coordinator of quality assurance for 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 freshly prepared working solution of Giemsa, made from well-prepared stock and diluted with water buffered to pH 7.2 is recommended to achieve optimal staining of malaria blood films. ***Giemsa stock solution procured for national programmes is standardized to minimize frequent adjustments to SOPs on staining.***

Staining with Giemsa stain can be done with a rapid (10% working solution) method or a slow (3% working solution) method. The rapid method is used in outpatient clinics and busy laboratories where a quick diagnosis is essential for patient care. The slow method is used for staining large numbers of slides, such as those collected during cross-sectional or epidemiological surveys and field research.

Some laboratories prefer to stain slides individually, even if there are relatively large batches, as this economizes the amount of Giemsa stain required. The volume of working Giemsa stain solution that is required, particularly for staining individual slides, should be determined precisely to obviate preparation of a large volume that may be used over a long time and possibly wasted.

### 3. SUPPLIES, MATERIALS AND EQUIPMENT

For 10% Giemsa working solution:

- Giemsa stain, transferred and filtered from the stock solution into a 25- or 50-mL bottle;
- buffered water, pH 7.2;
- a beaker or tube, clean, 5–10-mL capacity;
- a Pasteur pipette and
- Whatman filter paper, grade #1.

For 3% Giemsa working solution:

- Giemsa stain, transferred and filtered from the stock solution into a 25- or 50-mL bottle;
- buffered water, pH 7.2;
- a measuring cylinder, clean, 100–500-mL capacity;
- a Pasteur pipette and
- Whatman filter paper, grade #1.

### 4. SAFETY PRECAUTIONS

- Methanol (methyl alcohol) is flammable 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.
- 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
<p><b>A. Preparation of 10 mL of 10% of Giemsa working solution</b></p> <pre> graph TD     A([1. Place 9 mL of buffered water into a beaker or tube.]) --&gt; B[2. Filter the Giemsa stock solution through paper Whatman #1 and transfer to a 25 to 50 mL container.]     B --&gt; C[3. Add 1 mL of Giemsa stock solution.]     C --&gt; D([4. Use stain within 15 min of preparation, and discard unused stain.])   </pre>	<p><b>A. Preparation of fresh 10% Giemsa working solution from stock solution for rapid staining of a few slides.</b> About 3 mL of stain are required for each slide with a blood film.</p> <ol style="list-style-type: none"> <li>1. Place 9 mL of previously prepared buffered water, pH 7.2, into a clean beaker or tube.</li> <li>2. Filter the Giemsa stock solution through paper Whatman #1 and transfer to a 25 to 50 mL container.</li> <li>3. Using a clean, dry pipette, add 1 mL of Giemsa stock solution. Do <b>not</b> take the aliquot from the large bottle containing the Giemsa stock solution, to avoid contaminating it.</li> <li>4. Prepare the Giemsa working solution just before staining the blood film(s), and use it within a maximum of 15 minutes of preparation. Discard any unused stain.</li> </ol>
<p><b>B. Preparation of 100 mL of 3% Giemsa working solution</b></p> <pre> graph TD     A([1. Place 97 mL of buffered water into a measuring cylinder.]) --&gt; B[2. Filter the Giemsa stock solution through paper Whatman #1 and transfer to a 25 to 50 mL container.]     B --&gt; C[3. Add 3 mL of Giemsa stock solution.]     C --&gt; D([4. Use stain within 15 min of preparation, and discard unused stain.])   </pre>	<p><b>B. Preparation of fresh 3% Giemsa working solution from stock solution for slow staining of a batch of 20–100 slides</b></p> <ol style="list-style-type: none"> <li>1. Place 97 mL of previously prepared buffered water, pH 7.2, into a clean measuring cylinder.</li> <li>2. Filter the Giemsa stock solution through paper Whatman #1 and transfer to a 25 to 50 mL container.</li> <li>3. Using measuring cylinder or a pipette, measure 3 mL of Giemsa stock solution. Do <b>not</b> take the aliquot directly from the large bottle containing the Giemsa stock solution, to avoid contaminating it.</li> <li>4. Prepare the Giemsa working solution just before staining the blood film(s), and use it within a maximum of 15 minutes of preparation. Discard any excess stain.</li> </ol>

## 6. PROCEDURE NOTES

- Measuring cylinders, pipettes, containers and test tubes must be clean and dry before use.
- Do not make up a single batch of Giemsa stain for use or re-use throughout the day or longer. Giemsa stain quickly absorbs water vapour in the air, and, when diluted with de-ionized, distilled or any form of water, it rapidly loses its staining properties, so that slides stain poorly after just a short time. The iridescent scum on the surface of made-up Giemsa stain adheres easily to the blood film, making identification of structures difficult.

## 7. CALCULATING THE VOLUME OF GIEMSA STOCK SOLUTION AND BUFFERED WATER REQUIRED FOR STAINING INDIVIDUAL SLIDES.

Each blood film requires 3 mL of Giemsa stain working solution. The volume of Giemsa stock solution required for staining individual blood slides can thus be calculated from the formula below:

$$\text{volume of Giemsa stock solution required per slide} = \text{Giemsa concentration required} \times 3 \text{ mL}$$

The amount of buffered water (pH 7.2) required for staining a single slide can be calculated as follows:

$$\text{volume of buffered water per slide} = 3 \text{ mL} - \text{volume of Giemsa stock required per slide}$$

The 3 mL of Giemsa working solution are prepared by adding the volume of Giemsa stock solution required per slide plus the computed volume of buffered water at pH 7.2 required per slide.

### Example 1: Staining 15 slides with 10% Giemsa solution

The volume of Giemsa stock solution required for staining 15 individual slides with 10% Giemsa solution can thus be calculated as:

$$\frac{10}{100} \times 3 \text{ mL} \times 15 \text{ slides} = \text{volume of Giemsa stock solution required per slide}$$

$$(0.1 \times 3 \text{ mL}) \times 15 \text{ slides} = 4.5 \text{ mL}$$

Similarly, the volume of buffered water at pH 7.2 required for staining 15 individual slides with 10% Giemsa solution can be calculated as:

$$[3 \text{ mL} - (0.1 \times 3 \text{ mL})] \times 15 \text{ slides} = \text{volume of buffered water per slide}$$

$$[3 \text{ mL} - (4.5 \text{ mL})] \times 15 \text{ slides} = 40.5 \text{ mL}$$

Therefore, 4.5 mL of Giemsa stock solution should be mixed with 40.5 mL of buffered water to prepare the required amount of 10% Giemsa working solution for staining 15 individual blood films.

## 8. RELATED SOPs

MM-SOP-02: Preparation of Giemsa stock solution

MM-SOP-3a: Preparation of water buffered to pH 7.2

MM-SOP-3b: Preparation of water buffered to pH 7.2 with buffer tablets

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

MM-SOP 7a: Giemsa staining of malaria blood films

MM-SOP-11: General safety procedures in the malaria microscopy laboratory

## 9. REFERENCES

WHO. Basic malaria microscopy. Part I. Learner's guide. Second edition. Geneva: 2010.

Centers for Disease Control and Prevention. Laboratory diagnosis of malaria. Staining for malaria parasites. Atlanta, Georgia; 2013 ([http://www.cdc.gov/dpdx/resources/pdf/benchAids/malaria/malaria\\_staining\\_benchaid.pdf](http://www.cdc.gov/dpdx/resources/pdf/benchAids/malaria/malaria_staining_benchaid.pdf), accessed 14 December 2015).

## 10. DOCUMENT HISTORY

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