

PREPARATION OF GIEMSA STOCK SOLUTION

MALARIA MICROSCOPY STANDARD OPERATING PROCEDURE – MM-SOP-02

1. PURPOSE AND SCOPE

To detail the procedure for preparing a stock solution of Giemsa stain for routine staining of malaria thick and thin blood films.

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

Giemsa is the most commonly used stain for staining blood films for malaria diagnosis. It is available commercially as a ready-to-use product, but the quality varies according to the source. To ensure a consistently high-quality stain and a standardized product, it can be prepared by the reference laboratory of the national malaria programme and distributed to hospital or basic health laboratories in health facilities performing malaria microscopy. When simple rules are followed in the production of Giemsa stock, the stain produced at national or provincial level may be superior to that available commercially.

3. SUPPLIES, MATERIALS AND EQUIPMENT

To make 500 mL of Giemsa stain, the material required is:

- Giemsa powder or stain, 3.8 g (preferably Biological Stain Commission grade, to ensure a very good product of standard quality;
- absolute methanol, pure, high-grade, acetone-free, 250 mL;
- glycerol, high-grade, pure, 250 mL;
- methanol-cleaned solid glass beads, 3–5 mm in diameter, 50–100 pieces;
- a spatula or measuring spoon;
- weighing paper;
- a graduated cylinder;
- a glass or plastic funnel;
- a screw-capped, dark or amber glass bottle, clean and dry, 500-mL capacity (If not available, a chemically clean, dry, clear hard glass or polyethylene bottle of suitable size may be used, but should be wrapped in dark paper);
- an analytical balance capable of weighing to 0.01 g; and
- a shaker, if available.

4. SAFETY PRECAUTIONS

- Methanol and Giemsa stain are inflammable and highly toxic if inhaled or swallowed. Avoid contact and inhalation. When they are not in use, they should be stored in a locked cupboard or cabinet.
- 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
<pre> graph TD 1([1. Place about 50 beads in a bottle.]) --> 2[2. Weigh 3.8 g Giemsa powder and pour into bottle.] 2 --> 3[3. Gently pour in about 100 mL of methanol.] 3 --> 4[4. Tighten the screw cap on the bottle, and shake in a circular motion for 2–3 min.] 4 --> 5[5. Add 250 mL glycerol to the mixture, and shake for 3–5 min.] 5 --> 6[6. Add the remaining 150 mL of methanol.] 6 --> 7[7. Tighten the screw cap on the bottle.] 7 --> 8[8. Continue shaking for 2–3 min, six times on the first day.] 8 --> 9[9. Shake every day for at least 7 days.] 9 --> 10[10. Label the bottle clearly, and document in the quality control log book.] 10 --> 11([11. Tighten the screw-cap on the bottle, and store it in a cool place away from direct sunlight.]) </pre> <p>1. Place about 50 beads in a bottle.</p> <p>2. Weigh 3.8 g Giemsa powder and pour into bottle.</p> <p>3. Gently pour in about 100 mL of methanol.</p> <p>4. Tighten the screw cap on the bottle, and shake in a circular motion for 2–3 min.</p> <p>5. Add 250 mL glycerol to the mixture, and shake for 3–5 min.</p> <p>6. Add the remaining 150 mL of methanol.</p> <p>7. Tighten the screw cap on the bottle.</p> <p>8. Continue shaking for 2–3 min, six times on the first day.</p> <p>9. Shake every day for at least 7 days.</p> <p>10. Label the bottle clearly, and document in the quality control log book.</p> <p>11. Tighten the screw-cap on the bottle, and store it in a cool place away from direct sunlight.</p>	<ol style="list-style-type: none"> Place about 50 methanol-cleaned glass beads into a dark or amber bottle. Weigh 3.8 g of Giemsa stain powder on an analytical balance, and pour it into the bottle containing the beads through a funnel. Gently pour in about 100 mL of methanol, ensuring that all dry stain is washed into the bottle. Tighten the screw cap on the bottle. and shake it in a circular motion for 2–3 minutes to start dissolving the stain crystals. Add 250 mL glycerol to the mixture through the funnel, and shake again for 3–5 minutes. Add the remaining 150 mL of methanol to the mixture through the funnel, ensuring that the last of the methanol washes the last of the glycerol from the funnel into the stain mixture. Tighten the screw-cap on the bottle. Continue shaking for 2–3 minutes each about six times on the first day. Shake every day for 2–3 minutes each about six times for at least 7 days. A shaker maybe used, if available. Label the bottle clearly with the batch number, the name of the person who prepared the stock, date of preparation and date of expiry, and document in the quality control log-book. Tighten the screw-cap on the bottle to prevent absorption of water vapour from the air, and store in a cool place away from direct sunlight. <div style="background-color: #d3d3d3; padding: 10px; margin: 10px 0;"> <p style="text-align: center;">Giemsa stock solution Batch No.: 2015-01 Prepared by: First name Last name Date prepared: 17 Aug 2015 Expiry date: 17 Aug 2017</p> </div> <p><i>#2015-01 indicates the year prepared and the stock number.</i></p>

6. PROCEDURE NOTES

- The bottle should be tightly capped at all times to prevent absorption of water vapour and to avoid evaporation and oxidation of the stain by high humidity. If the bottle is tightly stoppered and free of moisture, the Giemsa stain is stable at room temperature for longer.
- Store in a dark glass bottle in a cool, dry, shady place, away from direct sunlight. If a clear stock bottle is used, wrap it in thick dark paper to avoid light penetration.
- Do NOT shake the stock Giemsa bottle for at least 24 hours before use to avoid re-suspending the precipitates, which will settle on blood films during staining and obscure important details during microscopy.
- Do NOT contaminate the stock Giemsa solution with water; even the smallest amount of water will cause the stain to deteriorate, making staining progressively ineffective.
- For daily requirements, measure and filter small amounts of stain into a tightly capped bottle (about 25–50 mL), so that the stock solution is less likely to be contaminated.
- Do NOT put or use a wet or soiled pipette into the stock Giemsa solution.
- Do NOT return unused or leftover stain to the stock bottle or to the bottle containing the working solution; stain that is out of the bottle must be used quickly or discarded.

7. QUALITY CONTROL AND DOCUMENTATION

- Perform a quality control check:
 - for every new batch or lot of stock solution prepared,
 - before using it as a working solution during Giemsa staining,
 - before sending it to laboratories for use and
 - in the field, after receiving it from a national reference laboratory.
- Record information in the quality control logbook. See MM-SOP 3c, *Quality control of Giemsa stock solution and buffered water*, for routine staining of malaria blood films.

8. RELATED SOPs

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

MM-SOP-04: Preparation of Giemsa working solution

9. REFERENCES

Storey J. Standard operating procedures for Giemsa malaria microscopy. 2012 (unpublished).

WHO. Basic malaria microscopy. Part 1. Learner's guide. 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, accessed 14 December 2015).

10. 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