World Health Organization

QUALITY CONTROL OF GIEMSA STOCK SOLUTION AND BUFFERED WATER

MALARIA MICROSCOPY STANDARD OPERATING PROCEDURE – MM-SOP-03C

1. PURPOSE AND SCOPE

To describe the procedure for quality control (QC) assessment of stock solutions of Giemsa stain and of buffered water (pH 7.2) for routine staining of malaria blood films. This SOP is applicable to both in-house preparations and commercially available reagents.

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

Monitoring of the performance of reagents is known as "quality control" (QC). In order to make an accurate diagnosis of malaria, it is essential that blood films be stained with good-quality preparations of Giemsa stock solution and buffered water at pH 7.2. These solutions should be tested before use, and, as good practice, a QC check must be performed:

- for every new batch or lot of stock solution prepared;
- before sending a stock solution to laboratories for use;
- In the field, after receiving a stock solution from a national reference laboratory;
- before using a stock solution to prepare a working solution of Giemsa stain and
- for every new batch of water buffered to pH 7.2 prepared.

The quality of the stain and buffered water should be checked on a thin blood film known to be positive for malaria. Blood films with *Plasmodium vivax* parasites are preferable in order to demonstrate the characteristic Schüffner dots. If these are not available, use a *P. falciparum*-positive blood film and examine it for Maurer clefts (if mature trophozoites are present). If a positive malaria blood film is not available, a negative blood film could be used to examine the colour and staining of red and white blood cells.

A supply of thin blood films could be prepared when fresh blood is available and stored at –20 °C or colder. The procedure is described in this SOP. Unstained films stored at room temperature will deteriorate over time.

Expected results

The stain should be tested at both 3% and 10% working solutions. Red blood cells will be seen as pinkish-grey, platelets deep pink and white blood cells (lymphocytes, neutrophils and monocytes) with a purple–blue nucleus and a pale cytoplasm. Eosinophils have coarse, bright purple–red granules in the cytoplasm, and neutrophils have finer, purple granules. Basophilic stippling in uninfected red blood cells is blue. The colours described above may vary slightly by batch of stain used and the character of the blood itself.

Malaria parasites should have a red or pink nucleus and blue cytoplasm. If *P. vivax* is used, the Schüffner dots should be seen as an even carpet of pink dots in the cytoplasm of red blood cells. If *P. falciparum* is used, Maurer clefts will be seen as unevenly distributed, coarse bodies in the red cell cytoplasm

The optimum staining time should be determined for each new batch of Giemsa stock stain. Staining of a blood film that is too dark or too pale may be corrected by adjusting the staining time. For example, if the cells appear pale and the stippling weak, longer staining may be required to obtain the correct result. The adjusted time should be recorded in the QC logbook and used for that batch of stock stain.

3. SUPPLIES, MATERIALS AND EQUIPMENT

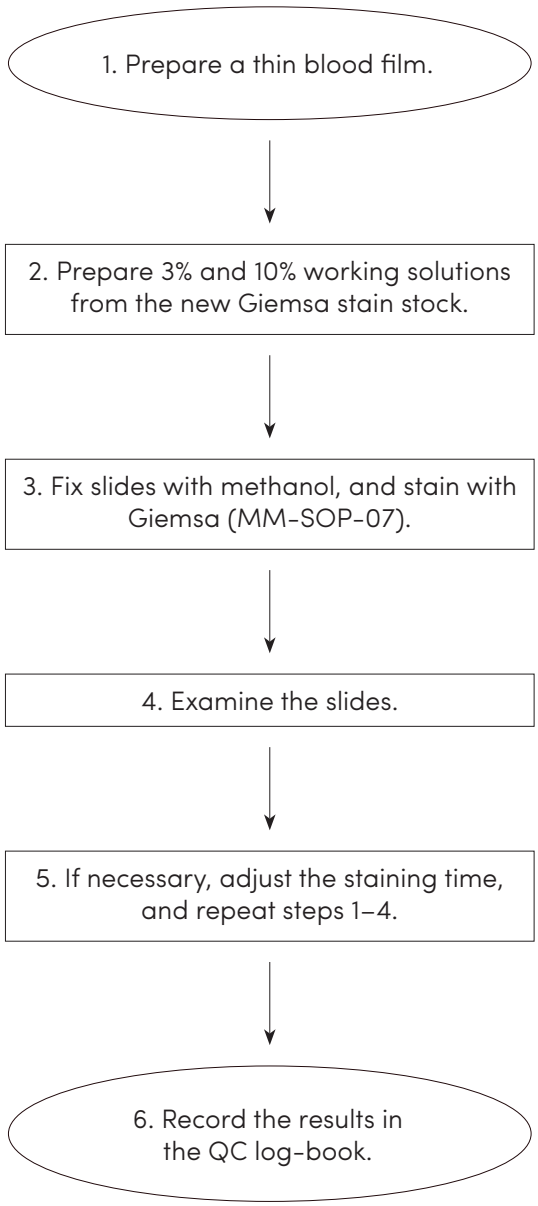
- desiccant (not containing cobalt chloride),
- a plastic box or ziplock bags, a freezer at –20 ˚C or –70 ˚C,
- pre-washed microscope slides and
- a cardboard box (for the microscope slides).

4. SAFETY PRECAUTIONS

Methanol and Giemsa stain are highly inflammable and are toxic if inhaled or swallowed. Avoid contact and inhalation. When they are not in use, they should be stored in a locked cupboard.

Universal precautions – including the use of relevant personal protective equipment such as gloves, safety glasses and a laboratory coat – must be practised. See MM-SOP-11: General safety procedures in the malaria microscopy laboratory

5. PROCEDURE

FLOW CHART	DESCRIPTION OF ACTIVITY
5.1. Quality control of Giemsa stain stock 	**5.1. Quality control of Giemsa stain stock** 1. Prepare a thin film of blood known to be positive for malaria, ideally containing *P. vivax* parasites. 2. Prepare 3% and 10% working solutions from the new Giemsa stain stock, as described in MM-SOP-04 (Preparation of Giemsa working solution) using water buffered at pH 7.2. **Use only buffered water that has previously undergone and passed a QC check.** 3. Fix the slide with methanol, and allow them to dry. Stain the slide according to MM-SOP-07 (Giemsa staining of malaria blood films) for both rapid (10%) and slow (3%) stains. 4. Examine slides to check for the quality of stain 5. If necessary, adjust the staining time, and repeat steps 1–4 until the expected results are obtained. 6. Record details, observations and actions in the QC log-book, with the name of the staff member who performed QC.

FLOW CHART	DESCRIPTION OF ACTIVITY
5.2. Quality control of water buffered to pH 7.2 1. Prepare a thin blood film. ↓ 2. Prepare 3% and 10% working solutions from the new Giemsa stain stock. ↓ 3. Fix slides with methanol, and stain with Giemsa (MM-SOP-07). ↓ 4. Examine the slides. ↓ 5. Record the results in the QC log-book.	**5.2. Quality control of water buffered to pH 7.2** 1. Prepare a thin film of blood known to be positive for malaria, ideally containing *P. vivax* or *P. ovale.* 2. Prepare 3% and 10% working solutions with the new buffered water stock, as described in MM-SOP-04 (Preparation of Giemsa working solution) at pH 7.2. **Use only Giemsa stain stock that has previously undergone and passed a QC check.** 3. Fix the slides with methanol, and allow them to dry. Stain the slides according to MM-SOP-07 (Giemsa staining of malaria blood films) for both rapid (10%) and slow (3%) stains. 4. Examine slides to determine the quality of staining. 5. Record the results, observations and actions in the QC log-book. Record the name of the staff member who performed QC.

FLOW CHART	DESCRIPTION OF ACTIVITY
5.3 Preparation of malaria-positive blood films for QC 1. Prepare blood films from malaria positive blood. ↓ 2. Fix thin blood film with methanol, and dry in air. ↓ 3. Pack the slides tightly in a slide box, and label. ↓ 4. Place the slide box in an outer box or ziplock bag with desiccant. ↓ 5. Store at –20 °C or colder. ↓ 6. Remove slides as needed, and place in a desiccator to thaw.	**5.3 Preparation of malaria-positive blood films for QC** 1. Using clean, washed slides, prepare some blood films (see MM-SOP-05) from a patient with falciparum or vivax malaria. Ideally, the film will show developing or mature trophozoites and, for *P. vivax*, gametocytes or schizonts. Allow to dry in air . 2. Fix the thin blood films with methanol as described in MM-SOP-07a (Giemsa staining of malaria blood films), and allow to dry in air. 3. Pack the slides tightly in a slide box front to back (the cardboard boxes in which slides are packaged are suitable), and label with the species and stages of parasites, the number of slides, date of preparation and the name of the staff member who prepared them. For example: 4. Place the box of slides into a plastic box or ziplock bag containing desiccant (not containing cobalt chloride). 5. Store at –20 °C or colder, ideally at –70 °C. Record the result in the QC log-book. 6. Remove slides as needed, and allow them to thaw or come to room temperature in a desiccator.

6. PROCEDURE NOTES

- Remember to prepare stocks of Giemsa and buffered water (pH 7.2) early enough that QC procedures can be completed in time.
- Store the Giemsa stock and buffered water in tightly stoppered, dark bottles in a cool place away from direct sunlight.
- Filter the small amount of Giemsa stain that will be used as a working solution and **not** the whole bottle.
- Do not return unused stain to the stock bottle or to the bottle used in daily routine. Once stain is out of the bottle, it must be used quickly or discarded.
- If buffered water tablets are used and the expected result is not obtained, the pH should be checked and adjusted, if there is the facility to do so. Otherwise, the buffer should be discarded and a fresh solution made. If the second buffered water solution fails the QC check, contact the national reference laboratory for further investigation. Use a different batch of tablets, if available.
- Check the pH of the buffered water at regular intervals.
- If you notice deterioration in staining, check the stocks of Giemsa stain and buffered water.
- Macroscopically, the blood film should look bluish–grey. A pinkish red colour is a sign of poor staining (too acid).
- When staining time is being optimized, a number of slides could be stained simultaneously but for different times. For example, with 10% stain, three slides could be stained together for 8, 9 and 10 min, and the staining time that gives the best results should be chosen for that batch of Giemsa stain stock.

7. DOCUMENTATION

- All QC procedures should be recorded.
- The records should indicate the date of testing, the batch numbers of the reagents, the date of preparation, the results of the QC test and any actions taken. The name of the person who performed the QC test should be included.
- All QC records should be kept.

Example record sheets are annexed to this SOP.

8. TROUBLESHOOTING

DESCRIPTION	POSSIBLE CAUSE	CORRECTIVE ACTION
Macroscopically, the thin blood film is reddish pink.	The pH of the buffered water used to make the working Giemsa stock too low, i.e. acidic.	Check the pH of the buffered water, and adjust it, if the facility is available. If not, discard the buffered water. If the pH of the buffered water is 7.2, check that all glassware is properly rinsed before use. Traces of detergent may alter the pH.
	The water used for rinsing is too acidic.	Use water buffered to pH 7.2 for rinsing.
Red cells have lysed or partially lysed on the thin blood film.	The thin film was not adequately fixed with methanol.	Use only good-quality, high-grade methanol. Store methanol in tightly stoppered bottled to avoid absorption of moisture from the atmosphere, which will affect its fixation properties.
The thick blood film has not haemolysed.	Red cells were fixed with methanol. The thick blood film has autofixed. Autofixation of thick films may occur if they are exposed to high ambient temperatures and humidity for prolonged periods. The thick film was heat fixed during incorrect drying, with e.g. a hairdryer or hot plate.	When fixing the thin blood film, avoid contact between the thick film and methanol, as methanol and its vapours will quickly fix the thick film. If blood films are not to be stained immediately then they should be stored in a desiccator containing silica gel (non-cobalt chloride) If rapid drying is required, dry the films at low heat from a hair drier for 5 s, at a distance of 30 cm.
Poor staining	Incorrect preparation of stock or working solution of Giemsa	Ensure that reagents are prepared correctly by following the SOP. Use an analytical balance to weigh Giemsa powder and graduated measuring cylinders to measure volumes accurately.

DESCRIPTION	POSSIBLE CAUSE	CORRECTIVE ACTION
Blood film covered in precipitate	Poor-quality stain used	Use only the stain recommended or supplied by the national reference laboratory.
	Poor-quality methanol or glycerol used	Use only high-grade methanol and glycerol.
	Giemsa stock not filtered	Filter small amounts of Giemsa stock immediately before making the working solution.
	Giemsa stock stored incorrectly	Store Giemsa stock in a dark or amber bottle, and keep it in a cool place away from direct sunlight. Keep the bottle tightly stoppered to prevent absorption of water vapour. Do **not** shake the bottle of Giemsa stock for at least 24 hours before use to avoid re-suspending the precipitates.
	The Giemsa stock may have been contaminated with water	Keep a small amount of Giemsa stock in a small bottle for daily use to avoid contaminating the whole stock. Do **not** put a wet or soiled pipette into the Giemsa stock solution. Do **not** return unused or left-over stain to the stock bottle or to the bottle containing the working solution; stain once out of the bottle must be used quickly or discarded.
	Blood film not rinsed correctly	If staining is done in a jar or tray, float the iridescent "scum" layer off before removing the slides.
	Working stain used after 30 minutes	Working stain should be made up just before it is required and used immediately. Excess stain should be discarded.
	Stain too old	Discard
Stain too pale	Insufficient staining time	Increase the staining time. The optimal staining time should be determined for each new batch of Giemsa stock.
	Working Giemsa stain filtered	Do not filter working stain. Small aliquots of Giemsa stock should be filtered before use.

DESCRIPTION	POSSIBLE CAUSE	CORRECTIVE ACTION
Stain too dark	Staining time too long	Decrease the staining time. The optimal staining time should be determined for each new batch of Giemsa stock.
Red blood cells are stained pinkish red.	The pH of the stain is too low (acidic)	Check the pH of the buffered water and adjust it to pH 7.2 if the facility is available. If not, discard the buffered water. If the pH of the buffered water is 7.2, check that glassware is properly rinsed before use. Traces of detergent may alter the pH.
	The water used for rinsing is too acidic.	Use water buffered to pH 7.2 for rinsing.
White blood cell nuclei are not dark purple.	The pH of the stain is too low (acidic).	Check the pH of the buffered water, and adjust it to pH 7.2 if the facility is available. If not, discard the buffered water. If the pH of the buffered water is 7.2, check that glassware is properly rinsed before use. Traces of detergent may alter the pH.
Schuffner dots, James dots or Maurer clefts are not visible.	The pH of the stain is incorrect.	If a pH meter or comparator is available, check the pH of the buffered water, and adjust it to 7.2. Otherwise, discard the buffered water. If the pH of the buffered water is 7.2, check that glassware is properly rinsed before use. Traces of detergent may alter the pH.
		Store clean, wrapped slides in a dry place. De-haemoglobinize thick

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https://www.yunbaogao.cn/report/index/report?reportId=5_27174