World Health Organization

EBOLA VIRUS INACTIVATION DURING STAINING OF BLOOD FILMS WITH GIEMSA STAIN

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

1. PURPOSE AND SCOPE

To describe the procedure for correct staining of malaria blood films with inactivation of haemorrhagic fever viruses (e.g. Ebola), in order to avoid work-acquired infections

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

Laboratory personnel who handle blood specimens from patients who may have been exposed to Ebola virus should assume that the specimens are contaminated with Ebola virus and should follow all safety precautions recommended for handling such specimens.

According to current knowledge, Ebola virus can survive for 48 h on a dried blood film. The modification of the Giemsa staining procedure described here allows safe handling of blood samples contaminated with Ebola virus and good-quality staining of malaria parasites.

3. SUPPLIES, MATERIALS AND EQUIPMENT

- Giemsa stain (3% solution) (See MM-SOP-04: Preparation of Giemsa working solution);
- 5%Triton X-100;
- a small container for Giemsa working stain;
- methanol, absolute, acetone-free;
- staining jars;
- a timer;
- a plastic pipette;
- a slide-drying rack, wooden;
- protective latex gloves (powder-free), disposable;
- a laboratory coat or gown;
- safety glasses and
- a class II biological safety cabinet, if available, or an isolation box.

4. SAFETY PRECAUTIONS

1. Use universal precautions. See MM-SOP 11: General safety procedures in the malaria microscopy laboratory.
2. Use appropriate personal protective equipment, including a laboratory coat, gloves and eye protection. See list of references for more details.
3. Do not collect finger-prick blood samples for malaria examination. Samples and specimens should be venous whole blood collected in EDTA-containing tubes.
4. Blood films should be prepared in the laboratory in a class II biological safety cabinet or isolation box.

5. Thick films and thin films must be prepared on separate slides. Avoid aerosol production during their preparation.
6. Allow films to dry completely before removing them from the biological safety cabinet.
7. Dispose of all contaminated materials in a pan to be autoclaved or in 10% sodium hypochlorite (bleach) for at least 5 min.

5. PROCEDURE

FLOW CHART

1. Prepare 5%Triton X-100 solution.
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2. Prepare two jars of 3% working Giemsa stain, add 5%Triton X-100, and mix. Label "thin films" and "thick films".
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3. Fix the thin film for 15 min in a jar with absolute methanol, and dry. Do **not** fix thick films.
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4.1. Pour 40–50 mL of Giemsa stain in buffered water into a third staining jar, add two drops of Triton X-100, and mix.
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4.2. Place slides with fixed thin smears and unfixed thick smears into the Giemsa stain jars with the corresponding labels "thin" and "thick" for 45 min.
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4.3. Remove each slide individually, and drain briefly on absorbent paper.
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4.4. Rinse each thin smear by dipping it three or four times into the solution of Giemsa stain in buffered water in the third jar.
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4.5. Place each thick smear slide into the buffered Giemsa stain for 5 min, dry upright in a rack, and examine at 100x.
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4.6. Discard the remaining 3% Giemsa solution.

DESCRIPTION OF ACTIVITY

1. Prepare 5% Triton X-100 solution with:
deionized water (warmed to 56 °C) = 95.0 mL
Triton X-100 = 5.0 mL
To dissolve the viscous Triton X-100 more easily, pre-warm the deionized water and slowly add the Triton X-100, swirling to mix. Do not shake.
2. Prepare two jars for 3% working Giemsa stain, and label one for thin films and the other for thick films. Add the amounts of 5% Triton X-100 indicated in the table below. Working Giemsa solution must be made freshly for each batch of smears.

Reagent	Thick blood film	Thin blood film
Giemsa buffered water (see MM SOP 03a and MM SOP 03b)	40.0 mL	40.0 mL
5% Triton X-100	2.0 mL	Two drops from a 1-mL pipette
Stock Giemsa stain (see MM SOP O2)	1.2 mL	1.2 mL
Staining time	45–60 min	45–60 min

Staining procedure for blood smears

3. **Fix thin films** for inactivation of Ebola virus for 15 min in a jar with absolute methanol. Allow to dry.
Do not fix thick films. Allow them to dry thoroughly before staining. Protect them from dust, insects, heat and fumes of absolute methanol.
4. **Staining procedure**

4.1. Pour 40–50 mL of water buffered to pH 7.2 into a third staining jar. Add two drops of Triton X-100, and mix. Use this solution for rinsing thin and thick films.

4.2. Place slides with fixed thin smears and with unfixed thick smears into the staining jars with the corresponding label, "thin films" and "thick films", and set the timer to 45 min.

4.3. At the end of the staining time, remove each slide individually, and drain briefly on absorbent paper.

4.4. Rinse each thin smear by dipping it three or four times in the buffered water in the third jar.

4.5. Place each thick film slide into the buffered water for 5 min.

4.6. Allow the smears to dry upright in a rack. After drying, the stained smears are ready to be examined; see MM-SOP 08: Microscopy examination of thick and thin blood films for identification of malaria parasites.

4.7. Discard the remaining unused 3% Giemsa solution. See MM-SOP 13: Management of wastes generated from malaria diagnostic tests.

6. PROCEDURE NOTES

Thin and thick blood films should be stained separately, because different amounts of the reagent 5% Triton X-100 are used. Exposure to the high concentrations of Triton X-100 indicated above for 45 min is essential to inactivate Ebola virus. Thin smears must be exposed to absolute methanol for at least 15 min to inactivate Ebola virus.

7. RELATED SOPs

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 04: Preparation of Giemsa working solution

MM-SOP 08: Microscopy examination of thick and thin blood films for identification of malaria parasites

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

MM-SOP 13: Management of wastes generated from malaria diagnostic tests

8. REFERENCES

Centers for Disease Control and Prevention. Guidance for malaria diagnosis in patients suspected of Ebola infection in the United States. Atlanta. Georgia; 2014.

WHO. Infection prevention and control (IPC) guidance summary. Geneva; 2014.

WHO. Personal protective equipment in the context of filovirus disease outbreak response. Geneva; 2014.

9. DOCUMENT HISTORY

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