

PREPARATION OF WATER BUFFERED TO pH 7.2 WITH BUFFER TABLETS

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

1. PURPOSE AND SCOPE

To detail the procedure for preparing buffered water **from buffer tablets** to pH 7.2 for use in preparation of Giemsa stain working solution for routine staining of malaria 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

Malaria parasites can be seen clearly under the microscope on properly stained blood films. The quality of staining depends strongly on the pH of the working stain solution. Use of buffered water at the correct pH (7.2) to dilute the stock stain solution helps to ensure good staining and ensures recognition of the specific features of malaria parasites. Buffered water must be prepared and quality-checked for the correct pH before staining blood films.

pH is the measure of the acidity or alkalinity of a fluid. It is based on a scale of near 0 (very acid) to 14 (very alkaline). Liquids that are neither acid nor alkaline are described as neutral, at pH 7.0. The pH of a liquid can be measured with a pH meter or with a colour indicator, such as the Lovibond comparator. Paper indicator strips can also be used, but they are rapidly affected by high humidity and become unreliable.

In this SOP, **use of commercially available buffer tablets** that indicate a specific pH when mixed in a fixed amount of distilled water (usually 1 L) is described. Buffer tablets do not have to be weighed and are useful in laboratories with limited facilities. They must, however, be kept in an airtight tube under dry conditions; otherwise, they rapidly absorb moisture and must be discarded. Some workers consider that the results of staining are inferior when buffer tablets are used, but there is no evidence to support this perception.

3. SUPPLIES, MATERIALS AND EQUIPMENT

- commercial phosphate buffer tablets for 1 L of water (pH 7.2);
- commercial distilled or deionized bottled water, 1 L;
- a graduated cylinder, 1-L capacity;
- a conical flask or beaker, 1-L capacity;
- a screw-capped glass bottle, clean and dry, of 1-L or 500-mL capacity and
- small tongs or tweezers.

4. PROCEDURE

FLOW CHART	DESCRIPTION OF ACTIVITY
<pre> graph TD A([1. Pour 1 L of distilled or de-ionized water into a flask or beaker.]) --> B[2. Place one buffer tablet into the flask or beaker.] B --> C[3. Mix by gentle swirling.] C --> D[4. Label the bottle of buffered water, and document the procedure in the quality control log-book.] D --> E([5. Use water buffered to pH 7.2 to prepare the Giemsa working solution.]) </pre>	<ol style="list-style-type: none"> 1. Measure 1 L of distilled or de-ionized water into a flask or beaker. 2. Using small tongs or a tweezer, place one phosphate buffer tablet into the flask or beaker. Be careful not to touch the tablet with your hand. 3. Mix by gently swirling the flask or beaker until the tablet is completely dissolved. 4. Label the bottle of buffered water as below, and document the procedure in the quality control log-book. <div style="border: 1px solid black; background-color: #cccccc; padding: 5px; margin: 10px 0;"> Buffered water pH 7.2 Date prepared: 17 Aug 2015 Expiry date: 24 Aug 2015 Prepared by: First name last name </div> 5. Use water buffered to pH 7.2 to prepare the working solution of Giemsa stain.

5. PROCEDURE NOTES

- The buffer tablets (pH 7.2) must be kept in an airtight container, away from sunlight and damp. They cannot be used if water vapour or moisture has reached them. Packed desiccant or silica gel (not containing cobalt chloride) should be placed in the buffer tablet bottles to prevent moisture.
- Always check the expiration date of the buffer tablets before use.
- Store the prepared buffered water in a cool place away from direct sunlight. Use of a dark bottle or a clear glass bottle wrapped in brown paper is recommended to prevent bacterial, fungal and algal growth.
- Check continuously for contamination.
- Do not keep buffer solution for more than 7 days to avoid a change in pH and to prevent contamination.
- Follow the manufacturer's guidance for use and storage of buffer tablets. Use only the amount of water specified by the manufacturer. Failure to do so will result in poor staining results.
- If facilities are available, check the pH of buffered water routinely before use, and record the result in the quality control log-book.

6. QUALITY CONTROL AND DOCUMENTATION

Perform a quality control check of every new batch of buffered water and before every use, and record the result in the quality control log-book. See MM-SOP 3c: Quality control of Giemsa stock solution and buffered water.

7. RELATED SOPs

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

8. REFERENCE

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

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

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