World Health Organization

PREPARATION OF WATER BUFFERED TO pH 7.2

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

1. PURPOSE AND SCOPE

To describe the procedure for preparing buffered water to pH 7.2 for use in the preparation of a working solution of Giemsa stain 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 correctly stained blood films. Buffered water must be prepared to dilute the stain before staining blood films. Using buffered water at the correct pH helps to ensure good staining.

This SOP has three components:

- preparation of water buffered to pH 7.2,
- preparation of 2% correcting fluid and
- checking and adjusting the pH of buffered water.

The pH indicates 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. National malaria programmes may recommend different kinds of pH meter or comparator. Laboratory staff should learn to use the device available in their laboratory.

In this SOP, use of the Lovibond comparator to measure the pH of buffered water is described.

Water can be made more acid or more alkaline by the addition of certain salts, called buffer salts. These are stored separately until combined in the correct proportions in a fixed volume of water to give the required pH. Buffer salts are weighed on a balance. It is important to ensure that they are stored correctly and cannot absorb moisture from the air; otherwise, they will not work.

Formulated tablets (buffer tablets) are commercially available, which indicate pH when mixed in a fixed amount of water (usually 1 L). Buffer tablets do not have to be weighed and are useful in laboratories with limited facilities. **A separate SOP (MM-SOP-03b) has been prepared for laboratories in which buffer tablets are used.** The tablets must, however, be kept in an airtight tube under dry conditions; otherwise, they rapidly absorb moisture and must then 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

3.1. Preparation of water buffered to pH 7.2

- an analytical balance accurate to 0.01 g (a two-pan trip balance is ideal). Various single-pan, electrically operated balances are available that are easy to use and suitable.
- filter papers, 11 cm in diameter;
- a conical glass flask, 1 L capacity;
- a glass beaker, 250 mL capacity;

- wooden spatulas (wooden tongue depressors are readily available);
- distilled or deionized water, 1 L;
- potassium dihydrogen phosphate (anhydrous) (KH_2PO_4);
- disodium hydrogen phosphate (anhydrous) (Na_2HPO_4);
- labelling paper and
- a screw-capped glass bottle, clean and dry, of 1-L or 500-mL capacity.

3.2. Preparation of 2% correcting fluid

- an analytical balance accurate to 0.01 g or better (a two-pan trip balance is ideal, or an electrically operated one-pan balance);
- filter papers, 11 cm in diameter;
- two glass-stoppered bottles, each of 100- or 150-mL capacity;
- potassium dihydrogen phosphate (anhydrous) (KH_2PO_4);
- disodium hydrogen phosphate (anhydrous) (Na_2HPO_4);
- distilled or deionized water, about 200 mL;
- wooden spatulas;
- two beakers of 250-mL capacity,
- one measuring cylinder of 100-mL capacity and
- labels.

3.3. Checking and adjusting the pH of buffered water

- buffered water in a conical flask,
- the two bottles of correcting fluid and
- a pH meter (portable or handheld) or a pH colour indicator and associated components.

If you are using a Lovibond comparator, you will need:

- two pH colour-indicator glass cells,
- one bottle of bromothymol-blue indicator and
- one measuring pipette, 1-mL capacity.

4. PROCEDURE

FLOW CHART	DESCRIPTION OF ACTIVITY
4.1. Preparation of water buffered to pH 7.2	**4.1. Preparation of water buffered to pH 7.2**
1. Weigh 0.7 g of potassium dihydrogen phosphate (KH_2PO_4).	1. Weigh 0.7 g of potassium dihydrogen phosphate (KH_2PO_4) on a two-pan trip balance: *Make sure that the pointer of the balance is set at zero by adjusting the balancing screw on the right arm. Place a filter paper in each pan; set the balance to zero, this time by moving the gram weight along the gram scale arm. Move the gram weight a further 0.7 g along the scale arm, ready for weighing the KH_2PO_4. Using a wooden spatula, place KH_2PO_4 on the filter paper in the left-hand pan until it reaches 0.7 g.*
2. Transfer the weighed KH_2PO_4 into a glass beaker. Add 150 mL of water. Stir with a spatula until the salt dissolves.	2. Transfer the weighed KH_2PO_4 to the glass beaker, add about 150 mL of water, and stir with a clean spatula until the salt dissolves.
3. Weigh 1 g of disodium hydrogen phosphate Na_2HPO_4.	3. Weigh 1 g of Na_2HPO_4. *Place a fresh filter paper in the left-hand pan. Reset the balance as before, but this time adjust the gram weight to 1 g. Using a clean, dry spatula, add* disodium hydrogen phosphate *Na_2HPO_4 to the right-hand pan, balancing the weight as described above.*
4. Add the Na2HPO4 to the solution in the beaker, and stir.	4. Add the Na_2HPO_4 to the solution in the beaker, and stir as in step 2.
5. Add the solution to the conical flask, and top up to the 1-L mark with water.	5. When the salts have dissolved, add the solution to the conical flask, and top up to the 1-L mark with water.
6. Pour the buffered water into a glass bottle. Label and document in the quality control log-book.	6. Pour the buffered water into a glass bottle. Label it clearly and document it in quality control log-book with the date of preparation, date of expiry and name of the person who prepared the buffer. Buffered water, pH 7.2 Prepared by: First name Last name Date prepared: 17 Aug 2015 Expiry date: 24 Aug 2015
7. Store buffered water in a cool place away from sunlight.	7. Store the buffered water for a maximum of 7 days, tightly stoppered in a cool place away from sunlight. Use of a dark or amber bottle is recommended.

FLOW CHART	DESCRIPTION OF ACTIVITY
4.2.1. Preparation of 2% Na_2HPO_4	**4.2.1. Preparation of 2% Na_2HPO_4**
1. Weigh 2 g of Na_2HPO_4.	1. Weigh 2 g of Na_2HPO_4.
2. Add to 100 mL of water in the beaker. Stir until the salt dissolves.	2. Add it to 100 mL of water in a clean beaker, and stir with the spatula until the salts have dissolved.
3. Pour the solution into a glass bottle, and label.	3. Pour the solution into one of the glass bottles, and label it "2% Na_2HPO_4". Write the date of preparation and the name of person who prepared the correcting fluid on the label.
4. Store the bottle in a cool place away from sunlight.	4. Store the bottle of 2% Na_2HPO_4 in a cool place away from sunlight.
4.2.2. Preparation of 2% KH_2PO_4	**4.2.2. Preparation of 2% KH2PO4**
1. Weigh 2 g of KH_2PO_4.	1. Weigh 2 g of KH_2PO_4 .
2. Add to 100 mL of water in a clean beaker, and stir until it dissolves.	2. Add it to 100 mL of water in a clean beaker. Stir with spatula until the salts have dissolved.
3. Pour the solution in a glass bottle and label.	3. Pour the solution into one of the glass bottles and label it "2% KH_2PO_4". Write the date of preparation and the name of person who prepared the correcting fluid on the label.
4. Store bottle in a cool place away from sunlight.	4. Store the bottle of 2% KH_2PO_4 in a cool place away from sunlight.

FLOW CHART	DESCRIPTION OF ACTIVITY
4.3. Checking and adjusting the pH of buffered water The following is the method for using the Lovibond comparator for measuring pH.	**4.3. Checking and adjusting the pH of buffered water** Check the pH of buffered water routinely before use. To adjust the pH, add small quantities of the correcting fluids to the buffer: 2% Na_2HPO_4 if the pH is below 7.2 (too acid) or 2% KH_2PO_4 if the pH is above 7.2 (too alkaline). Adjustments can be made as outlined below. The following is the method for using the Lovibond comparator for measuring pH.
1. Pour some of the buffered water to be tested into each of the pH colour-indicator glass cells up to the 10-mL mark.	1. Pour some of the buffered water to be tested into each of the pH colour indicator glass cells up to the 10 mL mark.
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2. Place one cell in the left-hand compartment of the pH colour indicator as the control cell.	2. Place one cell in the left-hand compartment of the pH colour indicator as the control cell.
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3. Pipette 0.5 ml of bromothymol blue indicator into the other cell, mix, and place the cell in the right-hand compartment.	3. Pipette 0.5 ml of bromothymol-blue indicator into the other cell, mix, and place the cell in the right-hand compartment.
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4. Turn the disc until its colour matches that in the right-hand cell.	4. Holding the pH colour indicator towards a clearly lit, white background, turn the disc until its colour matches that in the right-hand cell.
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5. Adjust the pH of the water in the conical flask by adding drops of the relevant correcting fluid.	5. Adjust the pH of the water in the conical flask by adding two or three drops of the relevant correcting fluid: Na_2HPO_4 to make it alkaline, KH_2PO_4 to make it acid. Stir with a clean spatula.
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4. Store bottle in a cool place away from sunlight.	6. Check the pH of the buffered water by repeating steps 1–5. Continue until the correct pH of 7.2 is reached.

5. PROCEDURE NOTES

- Many kinds of pH meter are available. It is recommended that staff learn to operate the kind available in their laboratory.
- It is best to store 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 continually for contamination.
- Do not keep buffer solution for more than 7 days to avoid change in the pH and to prevent contamination.
- Check the pH of buffered water routinely before use, and record it in the quality control log-book.

6. QUALITY CONTROL AND DOCUMENTATION

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

7. RELATED SOP

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