MINISTRY OF HEALTH - SULTANATE OF OMAN

SULTANATE OF OMAN
MINISTRY OF HEALTH

Malaria Diagnostics Operations Manual

Department of Malaria Eradication
Directorate General of Health Affairs, Ministry of Health HQ

Directorate General of Health Affairs

Department of Malaria Eradication

Department of Malaria Eradication
Directorate General of Health Affairs
P.O. Box 393, Postal Code : 100, Muscat
Phone : +968 24692715
Fax : +968 24692714

Contents

PREFACE

In spite of steady progress towards the elimination of malaria from our beloved Sultanate of Oman, we must remain alert to malaria in travellers and to preventing malaria outbreaks in isolated communities.

Having such skills and knowledge mean that any case of malaria managing to cross our international borders undetected, is discovered quickly so that our people are kept healthy and free from this terrible disease.

This Malaria Diagnostics Operations Manual focuses attention on the accurate diagnosis of malaria by using the well-proven system of Giemsa Malaria Microscopy (GMM). Called "The Gold Standard" of diagnosis it is carried out by staff trained to do this, at either the most modern hospital laboratory, or right out to the most remote Health Centre. However, this highly respected Gold Standard can only be maintained if every person involved with the diagnostic services carries out each step of the complex routine, correctly and with precision.

To achieve this level of expertise requires skill, knowledge and accuracy and it is essential that the procedures, when doing this work, are standardized and practised in the very same way by each person in the diagnostic services. Deviation from these standard operating procedures (SOPs) is unacceptable. There is no room for cutting corners or experimentation with the methods outlined here because these are the approved methods that MUST be followed.

Similarly, the accuracy of your work is regularly monitored and confirmed by the National Malaria Eradication Programme's Core Group of experienced supervisory staff through their quality assurance (QA) activities. This means that a proportion of your examined slides are re-examined by highly qualified personnel who confirm your diagnosis. The SOPs that cover the QA of examined slides (commonly called 'Cross-checking' in Oman) are included and form an important part of this Diagnostic Operations Manual.

So, it is with much pleasure that I introduce this manual as part of our continuing fight against malaria knowing that it will have an important place on your workbench as an aid to the diagnosis and monitoring of malaria in patients.

Signed by : **Dr. Majed Al Zadjali**
Director, Department of Malaria Eradication

1. INTRODUCTION

1.1 What are SOPs?

Standard operating procedures have their origins in training and the acquisition of skills that are standardized across a number of specialised subjects. In the medical services, for example, when a nurse gives an injection s/he will follow a series of procedures (Sandard operating Procedures, SOPs) that are more or less the same in hospitals and clinics throughout the world, no matter what the language in that place may be.

Called 'competency-based, or mastery learning', this kind of training takes place as a series of steps where the trainee learns to carry out specific skills (using the necessary knowledge) to the levels of competence required. Thus, in Giemsa Malaria Microscopy (GMM) workers will follow the SOP relating to the diagnosis of malaria in patients, to the highest level possible -- frequently referred to as the "Gold Standard".

In the past, not all training or daily work followed this meticulous routine and the need for a standardized approach in reaching the SOPs steps and standards as they relate to the Oman NMEP are now recognized and required.

Thus SOPs are a collection of internationally recognised instructions on how to carry out a particular activity (e.g. Examining blood films for malaria) following specific steps (e.g. Preparing the blood film for examination with a X100 oil immersion objective) and to the required standards (e.g. Carefully examine each oil immersion field until 200 fields have been examined).

The SOPs are usually produced in manual form, loose leafed so that any changes to the SOP can be updated easily and universally. One set is being issued to each Unit carrying out the activities. Deviation from the specific SOP is not allowed and ICA (internal competence assessment) and QA (quality assurance) activities regularly follow and evaluate whether the SOP is being adhered to or not.

1.2 Who are the SOPs intended for?

These SOPs are intended for health institution-based laboratory personnel involved in the routine diagnosis of malaria in patients reporting to Health Centres, Hospitals and Private Practitioner's throughout the Sultanate.

The titles and job descriptions of these personnel may differ, one from the other, but each person is nationally registered and certified competent to diagnose in patients malaria in Giemsa-stained thick and thin blood films.

In this series of SOPs, participant's must be competent in preparing from patient's a thick and thin blood film on the same slide, in accurately recording the patient's details on the slide label and linked with the designated register or record form, and the resulting results of the examination of the slide for malaria parasites.

This level of skills and knowledge is called the 'entry-level':

1. The sequence and procedures for the microscopical diagnosis of Giemsa- stained malaria parasites in patients attending OPD or In-Patient wards in Health Facilities or attending Private Practitioner clinics.
2. GMM for the diagnosis of malaria by stages and species in thick and thin blood films.
3. Methods of establishing parasite density for monitoring patient treatment, patient progress and regular reporting.
4. Methods for selecting slides for QA (cross-checking, or quality assurance) by supervisors and the reporting of discrepancies.
5. Identification of the appropriate syllabus when participants are in need of new and refresher training, based on the QA and ICA procedures.
6. Other matters regarding ICA (internal competency assessment) that is not a part of point 3 above.

1.3 Selection of the national Standard Operating Procedures

Until today the Oman National Malaria Eradication Programme (NMEP) has followed these long and well established training and working methods (SOPs) and laboratory-based diagnostic routines, all centred on a strong tradition of internationally-based knowledge and skills training, supervision and quality control.

To support these approaches a series of wall diagrams, information sheets, technical notes and Power-point Presentations were produced and extensively used for many years in training, supervision and quality assurance procedures. However, recent advances in GMM diagnostics and a general requirement to ensure that methods are standardized, as recognised SOPs, throughout the diagnostic services of the NMEP has dictated a review of the procedures now covered by this manual.

1.4 The SOPs Core Working Group

A Working Group consisting of core NMEP personnel has been formed to review existing SOPs as they relate to the daily diagnostic routine for malaria, to see where current practice differs and to decide whether changes are required to the existing SOP. This SOPs Working Group will meet at intervals to monitor whether changes to existing SOPs need to be initiated and made.

Members of the Working Group are:

	Job Title	Experience
Mrs. Farida Masoud Al Kindi	i/c NMEP Laboratory and Training	22 years
Mrs. Fatma Said Al Harrasi	Senior Laboratory Technician	19
Mrs. Laila Al Balushi	Malaria Microscopist	19
Mrs. Rabaa said Al Farsi	Malaria Microscopist	11
Cumulative years of GMM experience is about 71 years		

Doctor Majed Al-Zedjali, Director, NMEP, advises the group on questions of policy, feasibility, ethics, finance, planning and training. John Storey (WHO), has drafted the SOPs, facilitated and advises on questions of current diagnostic methods, internationally generated SOPs, internal competence assessment procedures and current external competence assessment approaches (ICA and ECA respectively) as well as training and distance QA and electronically-based learning and QA models.

Although seen as very complete and answering the current needs of the NMEP the SOPs included here are in a draft form and require the final recommendation of the Working Group and approval of the Director NMEP before wider circulation.

2. MALARIA LABORATORY MANAGEMENT

2.1 Technical responsibility

At each level of the diagnostic services, the medical laboratory technician (MLT), senior laboratory technologist or laboratory manager is responsible in ensuring that the GMM diagnostic process is carried out correctly following approved SOPs for that level. Any proposed deviations, from existing SOPs, can only be followed when approved by the Working Group on GMM-based SOPs. Observed lapses in SOP procedures, during routine QA activities, must be rectified quickly and correctly to avoid possible errors in diagnosis. This dictates that senior laboratory staff must have some background knowledge of the NMEP and how it functions. Figure 1 shows the general outline of GMM-based diagnostic services within the country.

In larger establishments, under the supervision of the laboratory manager, a staff member should be made responsible for the upkeep of the Patient Register, Daily Diary, Visitor's Book, selection of examined slides for 'cross-checking', as well as the upkeep of the Slide Bank, or Malaria Reference Library, and carrying out essential training when required.

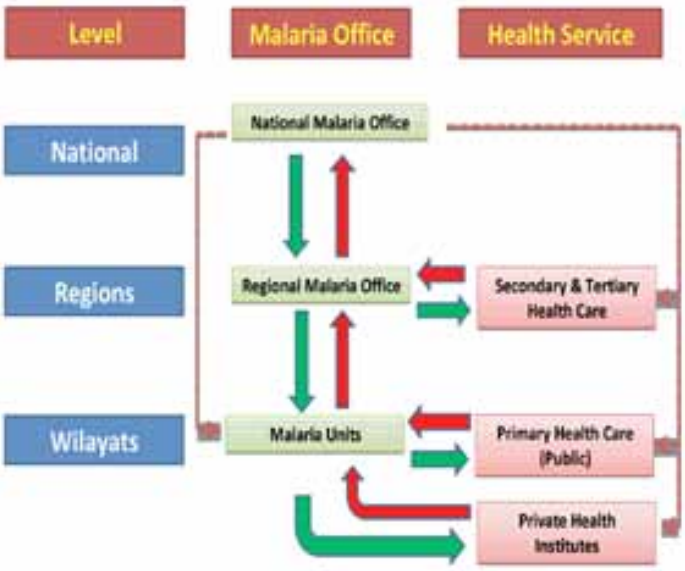


Figure 1: Oman: Diagnostic services at different levels, Oman *Courtesy Director NMEP*

Government policy requires private practitioners to submit positive and negative blood films from suspected malaria patients at treatment. Practitioner's can form quite a large portion of the treatment service and submit slides directly to a Government diagnostic unit, before finalising the diagnosis and treating. The rapid diagnostic test is not used. Others provide the service in their Clinic. This means that all patients are blood filmed with their read slides becoming part of the QA process. The coverage of such private clinics providing a diagnostic service is shown in Table 1.

Region	% of Private clinic that examine for malaria
Muscat	45%
Sharqiya	9%
N.albatinah	10.7%
S.albatinah	12%
Musandam	9%
Al-Dhahera	16%
Al-Dakhliya	16%
Al-Buraimi	6%
Dhofar	9%

Data courtesy Director NMEP

Table 1: Percentage of Private Clinics with a Malaria Diagnostic facility.

For convenience in these SOPs, personnel carrying out GMM are called 'Microscopist' no matter what their specific job title is, as many are polyvalent.

☒☒ Important to this SOP: Requirements before the collection of samples from patients

The collection and processing of samples from patients should be carried out only by staff who have completed the following training:

- Staff trained, or their abilities confirmed, to the required level in GMM, with certification or national registration
- Biohazard awareness is routinely and correctly practised.

SOP – GMM - 1

Cleaning and Storing Micro-Slides

SOP – GMM - 1

Cleaning and Storing Micro-Slides

SOP – GMM – 1 Cleaning and storing micro-slides

Diagnostic activities can use large numbers of glass micro-slides that must be correctly cleaned, wrapped and stored before work starts. Slides may be described on the manufacturer's box as 'cleaned' but they still need to be washed, dried and packed before they can be used for GMM.

☒ Important to this SOP: They must not be used straight from the manufacturer's packaging.

☒ Important to this SOP: Avoid using recycled slides for blood films.

GMM - 1.1 Equipment and materials

- New "Superior" quality glass, soda free, clear water clarity, micro-slides, size 25mm x 75mm 1mm thick with ground edges and one frosted end as a label.
- Two medium sized plastic bowls or basins.
- Domestic quality detergent liquid.
- Washing cloths or soft sponges (as required).
- A supply of clean, lint-free cotton cloths (the domestic kind used to dry glassware are best as they produce little lint).
- Am adequate supply of clean water.
- Sheets of clean paper cut to 11 cm x 15 cm.
- Empty slide boxes, the type in which new slides are supplied.
- Clear adhesive tape.
- In areas of high humidity: Desiccators or airtight containers charged with blue, when activated, silica gel.
- A locally made 'warm cupboard' i.e. a cupboard with tight-fitting doors and one 25-Watt bulb fitted per shelf.

Remember:

Poorly cleaned slides lead to:

- substandard blood films;
- imprecise microscopy;
- uncertain diagnosis;
- and affect the quality and reliability of slide materials and the well-being of the patient.
- Ensure slides are always properly cleaned, wrapped and stored

GMM - 1.2 Method

Cleaning is best carried out as a small group activity, as follows:

⇒ Remove the paper interleaves and briefly soak slides in a weak detergent solution.

⇒ With the washing cloth or sponge, clean each slide on both sides by rubbing the two surfaces between the forefinger and thumb.

⇒ Individually rinse slides in clean water to remove all traces of detergent.

⇒ Handling each slide by the edges, drain excess water and dry it using a clean, lint-free, cotton cloth.

⇒ Discard any slides that are chipped or scratched.

⇒ With the cut paper, wrap the dried slides in packs of 10 with the frosted labels at the same end.

⇒ The ends of the wrapper are folded and secured with adhesive tape. Place them, ready for use, in the cardboard boxes that the slides came in - secure the box with a rubber band.

⇒ Each box holds 10 packs of wrapped slides, making simple the calculation of the number of slides available.

⇒ Store wrapped, clean slides in a warm-cupboard, or silica gel charged desiccator or air-tight box, to ensure they remain completely dry until required.

⇒ For quality control (QC) each box should be labelled with details of the cleaning date and the name of the person(s) responsible for cleaning and wrapping the slides.

Remember:

In warm, humid climates fungal growths quickly appear on glass surfaces. To prevent fungal growths cleaned, wrapped slides must be stored in a warm cupboard, or in a desiccator charged with active silica gel.

SOP – GMM - 2

Making up:

(a) Giemsa stock solution and,

(b) Buffer water to pH 7.2

SOP – GMM - 2

Making up:

(a) Giemsa stock solution and,

(b) Buffer water to pH 7.2

GMM - 2.1 Making up a stock solution of Giemsa stain

The NMEP uses commercially available Giemsa stain and Buffer tablets at pH 7.2 as ready to use products and so this step of the GMM process are included here for reference, or in case the SOP needs to be changed at some time in the future.

☒☒ There are many important steps that can go wrong in this SOP.

☒☒ Read carefully through the bulleted steps on Giemsa and Buffer salts use, as they apply equally to items purchased commercially, or made up in the laboratory. Failure to do this may result in poorly stained blood films.

Other steps outlined in GMM – 2 can be skipped and kept for reference purposes.

☒ Note: Commercially available Giemsa stain (dry powder and solution ready for use) can be purchased as BSC grade - Biological Stain Commission. This assures a good standard quality of product at little extra cost[1].

Stock Giemsa is made as follows:

Formula

- Giemsa powder 3.8 g
- Methanol (pure) 250 mL
- Glycerol (pure) 250 mL
- Solid glass beads, 3-5 mm diameter: use 50 -100.
- One screw-topped, dark glass bottle of 500mL capacity, clean and dry.
- Analytical balance, weighing to 0.01 g
- Glass or plastic funnel

1 Webpage: biologicalstaincommission.org

Preparation steps and standards

(a) Place the beads into the clean, dry bottle.

(b) Weigh 3.8 g of the dry powder.

(c) Using the funnel for easy access, gently add the weighed stain powder to the bottle.

(d) Gently pour in half the amount of methanol ensuring the dry powder is washed into the bottle.

(e) Tightly stopper the bottle and shake it in a circular motion for 2-3 minutes to start dissolving the stain crystals.

(f) Using the funnel, add the glycerol and repeat the shaking.

(g) Pour the rest of the methanol into the bottle washing in also the remaining dregs of glycerol.

(h) Continue shaking the bottle for 3–5 minutes for 3 or 4 times in the first day.

(i) ☒ Repeat the shaking for the next 2 to 3 days when the stain is then ready to use. This preparation does not need filtering and the bottle should not be shaken any more. IT IS CALLED THE GIEMSA STOCK SOLUTION.

(j) Label the bottle clearly, including the name of the preparer and date, and store in a cool place away from direct sunlight.

(k) If there are only clear glass bottles they can be protected from light with a jacket made from thick brown paper to prevent light penetration.

(l) Although glass is preferred, polyethylene bottles can be used but should be fitted with a brown paper jacket.

Read the following carefully, it is required reading:

☒☒ With the Giemsa stock solution, it is important to remember the following:

- ❖ The screw stopper should be kept tightly closed at all times to prevent water vapour absorption by the stock stain.
- ❖ Once the stain is ready, the bottle should not be shaken any more;
- ❖ It does not need filtering.
- ❖ For daily use, decant stain into a 25 or 50 ml bottle labelled as Giemsa working solution.
- ❖ Do not contaminate either the STOCK GIEMSA SOLUTION OR THE WORKING GIEMSA SOLUTION with water, or leave the bottle open to absorb water vapour.
- ❖ Water must not be added to either of these two solutions; the smallest amount of water quickly

causes stain deterioration and progressively ineffective staining.

- ❖ The bottle must not be shaken before use. Shaking re-suspends the precipitates, which settles on blood films during staining and obscures important details during microscopy.
- ❖ Unused stain should not be returned to the stock bottle or to the bottle containing the working solution; stain once out of the bottle must be used quickly or discarded.
- ❖ Do not make up a single batch of Giemsa stain for use, or re-use, throughout the day, or longer. This is an unacceptable practice. Giemsa stain quickly absorbs water vapour in the air; and when diluted with de-ionized, distilled or any form of water, it rapidly loses its staining properties so that slides stain poorly after just as short a timeas 15-20 minutes.
- ❖ The iridescent scum on the surface of made-up Giemsa stain adheres easily

Buffered water to pH 7.2

To correctly stain blood films the Giemsa stain working solution MUST be diluted with water that has been buffered to pH 7.2. This can be done by balancing the water with buffer salts or with buffer tablets at pH 7.2.

☒☒ Even though the NMEP uses commercially obtained buffer tablets it is

Note on pH and Giemsa staining under operational conditions:

To achieve optimal staining quality Giemsa stain should be prepared by diluting the stock solution with water buffered to pH 7.2. ***Giemsa stock solution procured for a national programme must be standardized to minimize the need for frequent adjustments to staining SOPs which, on a national scale, raises a variety of other problems.***

Some commercially available Giemsa preparations require the pH to be varied above or below pH 7.2 to optimize the quality of that product. In such cases the national reference laboratory is responsible for determining the optimal pH and preparation conditions to achieve this, and then modifies SOPs for laboratories using this product. Clearly, in large national programmes this is an unacceptable process and adherence to a pH of 7.2 are strongly recommended.

Strongly recommended that you read through the rest of this SOP for the information it contains.

In well-stocked laboratories buffer salts should be weighed using an analytical balance. Ensure that buffer salt tablets are stored correctly and do not absorb water vapour.

Formulated buffer tablets are best used where the facilities in service level laboratories are limited. These tablets give a specific pH (7.2) when mixed with a fixed amount of water (100 or 1,000 ml as described on the label). Tablets must be kept in an airtight container, away from sunlight until use, to avoid absorbing water vapour. They cannot be used if water vapour has reached them.

To make up pH 7.2 buffered water

Note: Many laboratories are using pH 7.2 buffer tablets. If your laboratory is using buffer tablets then you can miss this section on making up buffered water and correcting fluid.

Equipment and supplies:

- Analytical balance, weighing to 0.01 g
- Filter papers, 11 cm in diameter
- One glass conical flask, 1 L capacity
- One glass beaker, 250 mL capacity
- Wooden spatulas (wood tongue depressors are usually available)
- Distilled or de-ionized water, 1 L
- Potassium dihydrogen phosphate (anhydrous) (KH_2PO_4)
- Disodium hydrogen phosphate (anhydrous) (Na_2HPO_4)
- None-absorbent cottonwool.

Procedure with steps and standards:

1. Set the pointer of the (two pan) balance at zero by adjusting the balancing screw on the right arm.
2. Place a filter paper in each pan; set the balance to zero this time by moving the gram weight along the gram scale arm.
3. Move the gram weight a further 0.7 g along the scale arm, ready for weighing the potassium dihydrogen phosphate.
4. Using a wooden spatula, place some of the potassium dihydrogen phosphate on the filter paper in the left-hand pan.
5. Transfer the weighed potassium dihydrogen phosphate to the glass beaker, add about 150 mL of water and stir with a clean spatula until the salt dissolves.
6. Place a fresh filter paper in the left-hand pan.
7. Reset the balance as before but adjust the gram weight to 1 g for the disodium hydrogen phosphate.
8. Using a clean, dry spatula, add the disodium hydrogen phosphate to the right hand pan balancing the weight as described in 4 above.
9. Add the disodium hydrogen phosphate to the solution in the beaker and stir as in step 5.
10. When the salts have dissolved, add the solution from the beaker to the conical flask and top up with water to the 1 L mark.

The buffer water is now ready for adjusting to pH 7.2 with the correcting fluid. Making up the 2% correcting fluids

Equipment and supplies:

- An analytical balance readable to 0.01 g
- 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 de-ionized water, about 200 mL
- Wooden spatulas
- Two beakers of 250 mL capacity
- One measuring cylinder of 100 mL capacity
- Labels

Procedure:

i. Follow steps 1 and 2 of the method for making buffered water, then move the gram weight a further 2 g along the scale arm.

ii. Weigh 2 g of disodium hydrogen phosphate and add it to 100 ml of water in the beaker; stir with the spatula until the salts have dissolved.

iii. Pour the solution into one of the glass bottles and label the bottle "2% disodium hydrogen phosphate".

iv. Repeat steps i to iii above only this time using 2 g of potassium dihydrogen phosphate and label the bottle.

Bottles should be stored in a cool place away from sunlight to avoid fungal and algal growths.

SOP-2.2 Checking and adjusting the pH of buffered water

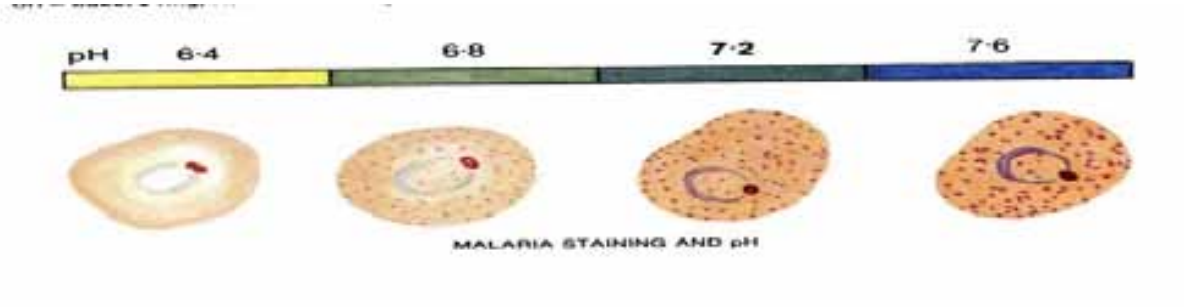
Buffered water should be checked for pH at the beginning of the working day. To adjust the pH, add small quantities of the correcting fluids to the buffered water – 2% Na_2HPO_4 if the pH is below 7.2 (too acid), or 2% KH_2PO_4 if the pH is above 7.2 (too alkaline). Adjustment follows the method outlined below:

Remember

Some programmes may use other systems to measure pH. There are many reliable kinds of pH meter in use. The important thing is to check the pH of the water used to dilute stains as regularly as possible. Water that shows a pH either side of pH 7.2 will result in progressively poor staining.

Equipment and supplies:

- The buffered water in a conical flask
- The two bottles of correcting fluids
- pH meter (e.g. colourimetric) with associated components (e.g. a 2/1H bromo-thymol-blue disc) and indicators (bromo-thymol-blue indicator)



Procedure:

a. Follow the instructions provided with the pH testing kit.

b. Adjust the pH of the water in the conical flask by adding small quantities of the appropriate correcting fluid.

SOP – GMM - 3

Blood filming patients

SOP – GMM - 3

Blood filming patients

Blood filming patients

In a busy laboratory one person may not always carry out the whole blood-filming routine. One person may do the first two steps with other steps being done by someone else. No matter how the laboratory routine is organized the following steps demonstrate the complexity of the routine. It is the responsibility of the laboratory manager, or senior technician responsible for routine activities, to ensure that all support staff, have the necessary skills and national registration to correctly do the following:

(a) Follow strictly all the established bio-safety procedures necessary when handling blood, blood contaminated articles and their eventual disposal.

(b) Record patient details correctly, ensuring they co-relate to information in the patient register.

(c) Ensure a calm and professional environment when taking blood from patient's by finger-prick, or by venipuncture.

(d) Make a thick and thin blood film on the same slide, or, for special work on separate slides. Note: Every worker should know the methods for making thick and thin films correctly.

(e) Staff must be aware that arrangements for screening patients and potential donor's, may be influenced by factors such as the location of communities and their cultural or religious beliefs, and to be able to act accordingly.

GMM – 3.1 Patients being blood filmed

Task: To record patient details in the designated form or register, make a thick and thin blood film on the same slide following established methods. The slide must be labelled on the frosted end with the patient's name, slide number, date, and other details as considered necessary and appropriate. This will allow easy tracing for follow-up, QA and other purposes. Using the thin film as a label is no longer encouraged.

Patient confidentiality must be maintained throughout.

Equipment and materials

- Latex protective gloves (powder free); allow a minimum three pairs per worker/day.
- Cleaned and wrapped glass micro-slides, with the frosted end used as a label.
- Paper sheet with printed templates for blood films, if considered necessary.
- Sterile lancets, one per person, plus 10%.
- Sharps container, see bio-safety comments.
- 70% ethanol. About 100 mL tightly stoppered bottle.
- Absorbent cotton wool – modest amount.
- Slide box or tray and cover, to dry slides horizontally, protected from dust, ants and flies.
- 4–6 clean, lint-free cotton cloths for drying slides.
- Record forms and/or register and pen.

Tips on Biosafety

Contamination with blood is a serious potential risk to worker and patient. Follow these precautions carefully:

- Wear protective gloves when handling blood and remove them before leaving the work area or when writing notes.
- Avoid getting blood, wet or dry, on fingers and hands.
- Carefully clean any cuts you may have and cover them with a waterproof dressing.
- Avoid pricking yourself with a possibly contaminated sharp instrument.
- Use lancets and syringe needles only once, disposing of them after use into a sharps container.
- After finishing a task, wash hands thoroughly with soap and water.
- If blood is accidentally spilt quickly wipe it away with cotton wool dampened with 70% ethanol.
- Delineate bench areas for slide preparation as ‘dirty’ and ‘clean’ areas for record management and slide examination (GM-Microscopy).
- Always keep these areas clean, tidy and strictly separated.

In most countries, strict regulations govern the disposal of contaminated lancets, needles, syringes, spoiled test-tubes, broken slides and other potentially contaminated materials. They are best discarded into a commercially available, yellow coloured “sharps container” that can later be correctly discarded following established SOPs. In the absence of a sharps container a bottle with a narrower mouth can be used and later incinerated. Never fill a sharps container to the rim. Waste should not be left at the peripheral facilities since they usually have poor disposal facilities. If possible, carry it back in easily identified, strong plastic bags/boxes for central disposal.

Syringe needles are NEVER used to prick fingers or as a substitute for the sterile lancet.

MAKING THE THIN AND THICK BLOOD FILMS ON THE SAME SLIDE [2]

1. Holding the patient's left hand, palm upwards, select the third finger from the thumb; clean the ball of the finger with cotton wool dampened with 70% ethanol to remove dirt and grease. Never use a baby's heel, a person's thumb or an ear-lobe. Dry the finger with a clean cotton cloth ensuring there are no strands of cotton left on the finger.

With a sterile lancet and a quick rolling action, puncture the ball of the finger, wiping off the first drop of expressed blood with a dry cotton swab.

2. Handling a slide by its edge apply gentle pressure to the finger and collect near the middle of the slide a single small drop of blood about this size for making the thin film

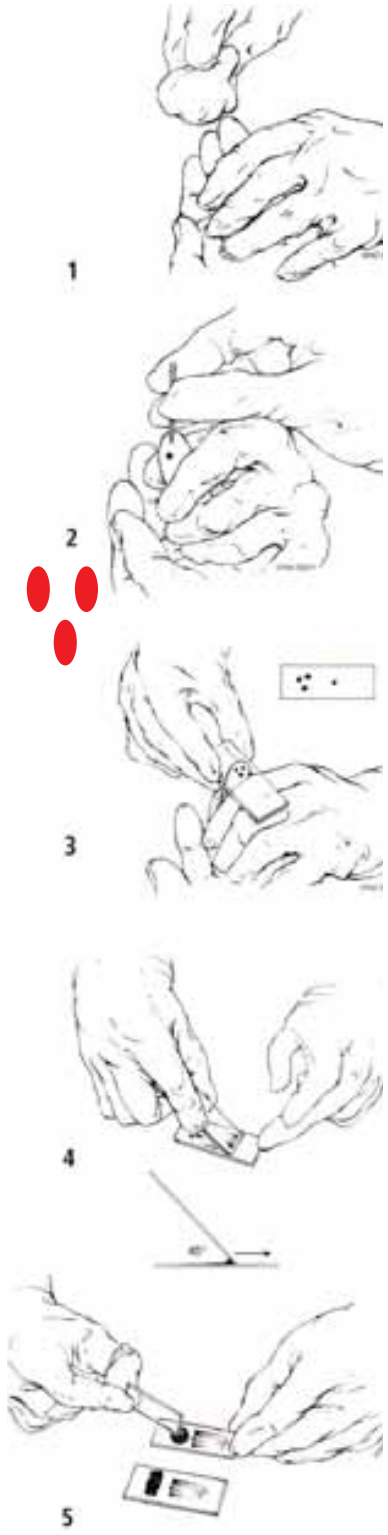
3. Quickly follow this up with three larger drops of blood placed on the slide about 1 cm from where the drop of blood for the thin film is. These are for making the thick film.

4. and 5. Thin film: Using a second clean slide as a spreader, with the slide with blood lying on a flat, firm surface, touch the small drop of blood with the edge of the spreader and allow it to run the length of the spreading edge. Holding the spreader tight to the surface of the slide and keeping it at an angle of about 45°, quickly and firmly push the spreader slide so that it forms a thin blood film behind it. Practice will help you to perfect this method.

Thick film: With the corner of the spreader slide join the three larger drops of blood stirring them in a circular motion and finally lifting the corner of the spreader slide from the middle of the newly made thick film. There is often a tendency to over stir the film while some workers prefer to make a rectangular thick film believing it to have a more even surface.

If not done before, the slides are labelled and then covered to prevent flies, dust and sunlight from reaching the films.

Note: In the past thin films have been used as a label but with the common availability of slides with frosted ends, is no longer recommended. Patient details can now be recorded with a soft lead pencil on the frosted end of the slide.



2 Illustrations courtesy of WHO.

SOP – GMM - 4

Staining blood films with Giemsa

SOP – GMM - 4
Staining blood films with Giemsa

GMM - 4.1 Staining the blood films

For malaria microscopy Giemsa stain is the stain of choice. If standard pH 7.2 buffered water is used throughout, and other simple rules followed, then staining of a high consistently high quality will result.

☒☒ Important to this SOP: Following simple rules like:

- Only making the stain up just before use;
- Do not shake the bottle at any time as it displaces stain residue;
- Gently wash the iridescent scum off the slide at the end of staining before washing the stain off;
- In the staining process always use water buffered to pH 7.2

Care with these will give you continuously good results.

Giemsa is the most reliable of the alcohol-based Romanowsky group of stains. Others in the group are water-based and mostly used as a rapid stain. They do not have the reliability and consistency of results as Giemsa.

Two methods are used for staining blood films, (i) the rapid (10%) method, where a quick diagnosis is needed and which takes about 10 minutes for a result; and (ii) the slower and more economic (3%) method that takes 30 minutes and is best used for larger numbers of slides from surveys or for training or slide bank collections, where diagnosis is not urgent but the stained result is required to be excellent.

(i) The rapid (10%) method of staining

This method is used to stain single, or just a few slides together, and when a rapid result is required. It is commonly used in laboratories and health facilities where GMM routinely takes place.

Equipment and Materials

- Giemsa stain, decanted from the stock solution into a 25 or 50 ml bottle.
- Methanol. 100 ml [3]
- Absorbent cotton wool or gauze.
- Test tubes of 5ml capacity.
- Distilled or de-ionized water buffered to pH 7.2. About 1 Liter.
- Pasteur pipette with rubber teat, or the plastic product.
- Curved plastic staining tray or plate, or a staining rack that fits over the sink or basin.
- Slide drying rack.
- Timing clock.
- One or two shallow basins to place the staining racks in or over.

Note:

- ✓ Thick blood films must be completely dry before being stained. Otherwise there is risk of the blood film floating off during staining.
- ✓ If possible dry slides overnight in an air-tight container with a desiccant
- ✓ If in a hurry thick films can be carefully dried with a hair-dryer set at very gentle heat. The danger is that too much heat can be used and the thick film heat-fixes and is useless. Practice and careful observation will lead to doing this correctly.
- ✓ Storage without staining for even just one or two days in hot, humid conditions can result in auto-fixation and the thick film being useless for microscopy. Although slides can be stored in a desiccator in the deep freeze for many months, even years, without affecting the staining.

3 Methanol (methyl alcohol) is highly toxic and flammable; it can cause blindness and even death if swallowed in any quantity. When not in use it should be stored in a locked cupboard.

Procedure

(a) Fix the thin film by dabbing it gently with a pure methanol dampened pad of cotton wool, or, dip it briefly into methanol. Note: If methanol, or methanol fumes, make contact with the thick film, the thick film will quickly fix and be useless.

(b) Using a small container to hold the prepared stain, make up a 10% solution of Giemsa stain in buffered water, mixing it well. Each slide will need about 3 - 4 mL of stain to cover it. Three drops of Giemsa stain from the Pasteur pipette added to each mL of buffered water makes approximately a 10% solution. The diluted Giemsa should be prepared immediately before staining and any excess stain discarded. Dilute Giemsa stain must be used within 10 minutes after preparation and then discarded.

(c) Slides should be placed face down on the curved staining tray, or face upwards on the staining rack; the one being used in placed on the rim of the plastic bowl, placed in the sink for easy washing of the slide(s).

(d) With the staining tray, the stain is poured gently between the slide and staining tray until each slide is covered with stain, or, using the staining rack, the stain is poured gently onto each slide lying face upwards on the rack.

(e) Stain the films from 8 to 10 minutes. Stains vary in quality from batch to batch so the optimal staining time should be determined by first staining a series of slides for varying time periods and establishing the best staining time that way.

(f) When the time has elapsed, working quickly gently flush the stain from the slide by tap water over the slide. Do not pour stain directly off the slide otherwise the metallic-green surface scum sticks to the film, spoiling it for microscopy.

(g) When the stain has been washed away, place the slides, film side down, in the drying rack to drain and dry.

(ii) The slow (3%) method of Giemsa staining

This method is excellent for staining slides in bulk, for preparing teaching material, survey work or for slide bank material. The method performs best when slides have been dried for 24 hours or overnight. This is especially so if the blood has been treated with EDTA. EDTA treated blood does not adhere well to slides and has a tendency to float off during staining; it benefits from a longer drying period.

Note:

- ✓ With bulk staining, if possible, first test a representative sample to establish the staining properties.
- ✓ This is particularly necessary with films made with EDTA treated blood, or other anti-coagulants, as they can adversely affect the pH and staining properties.
- ✓ Similarly, EDTA treated blood does not adhere well to the micro-slide and needs to be thoroughly dried before staining.
- ✓ Stain about 4 thick films in a 3% fresh Giemsa solution and evaluate the staining microscopically.
- ✓ If necessary, adjust the pH of the buffered water, based on the microscopically visual quality of the staining, although this is not usually necessary.
- ✓ Staining that is too pink requires the buffered water to be more alkaline; too blue to be more acid. Trouble taken over this pays dividends in the end product of a mass staining exercise.
- ✓ If a thin film is also on the slide then it must first be methanol fixed before staining, as previously described.

Method used for staining 20, or more, thick blood films at a time.

Equipment

- ❖ Giemsa stain
- ❖ Methanol pure
- ❖ Absorbent cotton wool or gauze
- ❖ Staining troughs to hold 20 slides placed back to back, depending on the size of the staining trough. Note: Coplin jars are not good for staining blood films with Giemsa; they are messy to deal with and surface scum usually ends up on the films.
- ❖ pH 7.2 buffered water, adjusted, based on the recommended test run
- ❖ Measuring cylinder, capacity 100 mL–500 mL
- ❖ Measuring cylinder, capacity 10 mL–25 mL
- ❖ Flask or beaker, capacity will depend on the amount of stain to be made up
- ❖ Timing clock
- ❖ Slide drying racks

Procedure: Steps and standards

In advance: Calculate the total amount of stain required for this staining session. Do so by covering 20 blank slides in a staining trough with water, measuring the amount of water the trough takes. The total amount of stain required is calculated by multiplying the number of troughs, filled with slides, by the amount of stain each trough requires.

☒☒ Commercially available bottled water ranges from pH 6.8 to pH 8. Making stain up or rinsing slides with these products can give levels of colour from decidedly red to deep mauve. Neither extreme is what we want. Keep to pH 7.2

i. Place slides in a staining trough, back to back, with thick films at one end of the trough and thin films at the other. The stain, and water when rinsing, will be introduced at the thin film end so there is less chance of thick films floating off.

ii. Prepare a 3% solution of Giemsa stain by adding 3 ml of Giemsa stock solution to 97 ml of pH 7.2 buffered water, or its multiples.

iii. Ensure the stain and buffer are well mixed.

iv. Avoid pouring stain directly on to the thick films by gently pouring the stain into each trough at the end where the thin films are.

v. Stain for 30 minutes - experience will select the best time.

vi. At the thin film end of the trough gently pour in clean water and float off the iridescent “scum”. A less satisfactory method is to gently immerse troughs into a basin of clean water - but avoid slides picking up the iridescent scum when removing the troughs from the basin.

vii. Rinse the slides with clean water. The pH of the water used for rinsing is important as acidic water will de-stain films while rinsing whereas alkaline water (pH 7.2) maintains the quality of staining.

viii. After staining, place slides film side down in a drying rack to dry. Ensure wet thick films do not touch the edges of the rack and get scraped off.

ix. When completely dry, films should be packed, face to back, wrapped in clean, dry paper and stored in a desiccator charged with activated (blue) silica gel.

☒☒ The transfer of parasites during staining

Parasite transfer between slides during staining has been shown to happen, especially with larger stages such as gametocytes or schizonts. The average worker need not worry about this though as the mass staining of blood films made from one donor ensures that if parasite transfer takes place it can make little difference to the result.

☒☒ More importantly, dirty and improperly cleaned and dried utensils have a small risk of retaining free-floating parasites that can later adhere to a film during new staining. In order to minimize these risks, never stain together large numbers of slides from different donors. Most of all discard used staining solutions and thoroughly clean the glassware and staining troughs between staining activities.

Care of glassware and measuring utensils

Measuring cylinders, pipettes, staining troughs and beakers must be thoroughly clean and dry before use. Staining blood films using dirty utensils will mean an unsatisfactory result.

After use, immediately rinse used utensils with clean water to remove the free stain and soak it in a detergent solution before final washing. Thoroughly rinse and dry.

Note: ☒☒ Detergent left on poorly rinsed glass and plastic-ware will subsequently alter the pH and affect staining quality.

SOP – GMM - 5

Examining thick and thin blood films for malaria parasites

SOP – GMM - 5
Examining thick and thin blood films for malaria parasites

Note ☒☒ This is not a training manual. In training manuals the lesson for examination of thin films for malaria parasites comes before lessons on the thick film examination. Therefore to go into this SOP (GMM-5), and beyond, you should be a registered Microscopist working with the NMEP and fully familiar with malaria parasite identification..

☒☒ There will be a number of coloured plates and Bench Aids as a part of this SOP to assist with identification of parasite stages and species.

☒☒ **Throughout this series of SOPs, please demonstrate your professionalism by fully respecting patient confidentiality.**

Remember

We use thick blood film microscopy to determine more quickly the presence and density of malaria parasites.

When parasites are present, then use the thin film to aid species diagnosis and naming the stages present.

Less experienced Microscopists may have difficulty with differentiating in thick films between:

- The later mature trophozoites and gametocytes of *P. vivax*.
- Mature *P. malariae* trophozoites and rounded *P. falciparum* gametocytes.
- The late trophozoite and gametocyte stages of *P. malariae*.
- Mixed infections of two or more species can be difficult to identify.
- The fact that *P. falciparum* infections normally consist of small rings with no obvious effect on the host red cell, can mean that other species, in their very early stages, can slip through and be mistaken for *P.falciparum*.
- An exception to this rule is with *P. falciparum* infections in semi-immune individuals who may have some larger rings and demonstrate Maurer's clefts in a well-stained thin film.
- In daily routine situations where the instruction is to record the presence of gametocytes it

should be confined to easily diagnosed *P.falciparum*.

- The potential problem of *P.knowlesi* identified in traveler's from SE.Asia while remote, is nonetheless, an area where awareness of the possibility, and knowledge of differences in morphology would prove valuable. The important point is that the Microscopist recognizes the blood film as positive and ensures the patient is treated as soon as possible. These latter actions the come within the confines of the clinician (and a possible, subsequent confirmation by PCR if considered appropriate).

☒☒ If a worker is finding difficulty differentiating between some parasite stages and/or species then s/he must be given a reasonable amount of tutoring time to resolve this problem. However, confidence in the Microscopist's abilities and competence (to call a slide positive or negative) remains with the supervisor or Laboratory Manager who must ensure that s/he is satisfied with the individual Microscopist's performance. The need for additional and appropriate training, or retraining, is at issue here and much will depend on the attitude from the Central Level as well as the Laboratory Manager's own work experience - as well as how important routine malaria diagnosis is in that laboratory.

Equipment and Materials:

- A binocular microscope fitted with a mechanical stage, paired x10 oculars and a x10, x40 and x100 objectives.
- An objective marker may be fitted to highlight specific objects.
- A mains power supply, battery, or solar powered microscope lamp.
- The slides to be examined stored in a wooden slide box with lid.
- A second slide box to hold on one side the positive slides identified and on the other the negative slides, following their examination
- Immersion oil of an approved quality and refractive index
- Tally counters, preferably the multi-counter type; these are more robust and also allow differential counting of WBCs in polyvalent situations.
- record forms,
- Pen and pencil.
- Electronic calculator.
- Diagrams and photomicrographs of parasite stages and species – as contained in Learning Unit 8 and 9 of the WHO manual, Basic Malaria Microscopy and in the Bench Aids for Malaria Diagnosis one set of each being recommended per laboratory.
- A laboratory timer.

EXAMINING THE BLOOD FILM

The method:

(a). Scanning the film for quality, other parasites and the 'best part':

Microscopist's will have received training in this activity during the Basic Malaria Microscopy Training Course, certificate examination and subsequent national registration, and so these instructions are included more to confirm the established methodology rather than any new instructions.

1. Switch the lamp on and make sure that the x40 objective is in place over the stage. Note: The film can only be scanned after the immersion oil has been put on to the film.
2. Place a drop of immersion oil on the thick film so that it covers the area considered suitable for the examination.
3. Place the slide onto the stage with the slide label to the right of the stage and the blood film under the x40 objective.
4. Focus on to the film.
5. Once in focus, scan the film to establish the following:
   - ❖ The blood film is suitable for examination with good thickness, good staining, good lysing and no wash-offs that may interfere with a complete examination?
   - ❖ There are no unusual factors such as an uneven distribution of WBCs, or the presence of quantities of debris or artefact that will interfere with the examination.
   - ❖ Record the presence of other possible parasites such as microfilariae, trypanosomes or unusual numbers of WBCs such as with Eosinophilia.

This should take no more than half a minute and scanning the film in this way helps to select the best part of the film to start the examination.

(b). Examining the blood film for malaria parasites:

Note: Blood films are examined for 200 oil immersion fields; and, if no parasites are found the slide is declared negative for MPs. This takes between 8 – 10 minutes depending on the conditions. It may take longer if the parasite density is low and finding confirmation difficult.

6. When the 'best part' of the film has been selected turn the objective to the x100 (oil immersion) and start the examination.
7. If selected well there will be about 12 -15 WBCs per oil immersion field.
8. Scan each field for malaria parasites ensuring to continually focus through the depth of the field with the fine adjustment. Failure to focus correctly may mean that parasites that are currently out of the depth of focus are missed.

9. Most skilled workers first scan the edge of the field, focusing as they go, and follow that up by focusing on the centre of the field. When a suspected parasite is seen it is confirmed by re-focusing on it and confirming or rejecting it as a parasite. Usually this is a straightforward procedure.

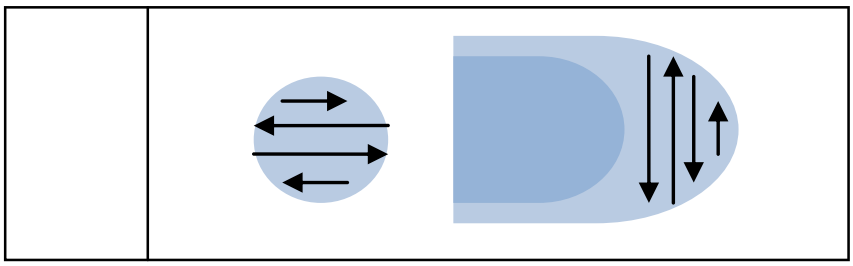
10. Carry on in this way, moving the fields over the film in the direction as shown in the diagram, until 200 oil immersion fields have been examined. This usually takes between 8 - 10minutes; the fields can be counted on counter.

11. If no parasites have been seen in 200 oil immersion fields then the slide is declared MP Negative or 'no parasites seen in 200 oil immersion fields'.

12. When a parasite is seen, first confirm, and then establish the stage and the species and, if it is part of the routine, carry out a count to establish the parasite density.

13. Establishing parasite density is at present in Oman done only with patients who are warded and is so designed to assist the physician with monitoring patient progress. Parasites are counted against WBCs until 200 WBCs have been counted. This is dealt with in detail below.

14. The stage(s) of the parasite is usually determined quite easily in the thick film. Where P.vivax and P.falciparum infections are present in approximately equal numbers, species thick film diagnosis should be straightforward providing the staining is of the correct standard. Deciding the stages and species may prolong the examination time beyond the 10 minutes or so normally allocated. Do not be too influenced by this as accuracy and the patient's well-being is what is important.



Patterns of examination of the thick and thin films

Following examination, the slide is placed in the designated slide box for storage. Immersion oil can be gently cleaned off with a cotton-wool pad soaked in xylene.[4] Positive and negative slides are separated for later selection for QA.

Examination, for species confirmation, in the thin film follows that shown in the 'Pattern of Examination diagram' and experience and attention to standardization will help staff become experienced.

4 Because Xylene is known to have carcinogenic properties, cleaning should take place wherever possible in a fume cabinet.

Recording the result of the examination:

Results of the examination are recorded in the appropriate registers and forms.

At present there is no standardized way to record the result of the examination. Many methods exist but it is essential to establish one method in a national programme that can be understood and followed by all. The following are the most commonly used methods and staff will be instructed which ones are to be used in the MMEP:

Positive for malaria parasites:	MP positive; MP
Negative for malaria parasites:	MP negative, MP neg
Positive for P.falciparum ring:	Pfr
Positive for P.falciparum ring with schizont:	Pfr,sch
Positive for P. falciparum gametocytes:	Pfg
Positive for P.vivax:	PV
Positive for P.malariae:	PM
Positive for P.ovale:	PO

The parasite count:

The parasite density of a positive blood film is usually required to:

- Assist the treating clinician of the density of the infection, the seriousness of the patient's illness, and through subsequent blood parasite density counts and follow-up, to monitor the patient's response to treatment.
- In *P.falciparum* infections the parasite count is important as this species is potentially life-threatening.
- Health officers should be aware of the severity of cases in communities in their district.
- Determination of density of infections is important in cross-sectional and epidemiological and special investigations.

At present density counts in Oman are confined to warded patients, or on special request. As the follow-up and QA system becomes more sensitive counting may be extended to other patients but that is not current policy.

Density count method: Parasites per micro-litre

Method

On the counter count the number of parasites seen per field, against the number of WBCs until reaching the number of parasites to 200 WBCs.

When completed, the number of parasites relative to the number of white blood cells is calculated and expressed as 'parasites per microlitre of blood' following the simple formula:

$$\frac{\text{Number of parasites counted} \times 8000}{\text{Number of WBCs}} = \text{parasites per microlitre}$$

In mixed infections of two species or more, it is usual to count all asexual parasites together and to express the result as, for example, Pf + Pv = 593 parasites against 200 WBCs x 8000.

The Plus, Plus System of Counting parasites

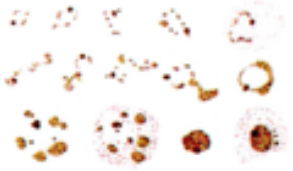
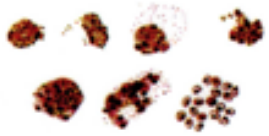
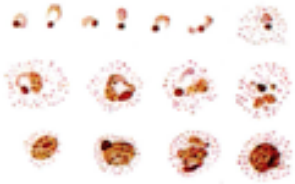
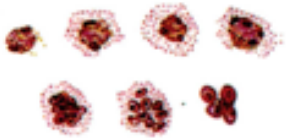
This is a sytem that still sees favour in areas where the parasite rate in communities is high, or where parasite densities are high, or where the heavy workload allows little time for the parasite density (per micro-litre method) to be used. In most cases providing the simple formula is correctly followed this is a perfectly acceptable method. In the Oman NMEP, the "Plus, Plus system" continues to be used and is a comparatively easy way to record the number of parasites seen in a positive blood film as described below:

\+ 1 – 10 parasites per 100 thick film fields

++ 11 – 100 parasites per 100 thick film fields

+++ 1 – 10 parasites per one thick film field

++++ more than 10 parasites per one thick film field.

Species		Stage of parasite in peripheral blood		
		Trophozoite	Schizont	Gametocyte
Plasmodium falciparum	Young, growing trophozoites and/or mature gametocytes usually seen.	 *Size:* small to medium; *number:* often numerous; *shape:* ring and comma forms common; *chromatin:* often two dots; *cytoplasm:* regular, fine to fleshy; *mature forms:* sometimes present in severe malaria, compact with pigment as few coarse grains or a mass.	 Usually associated with many young ring forms. *Size:* small, compact; *number:* few, uncommon, usually in severe malaria; *mature forms:* 12–30 or more merozoites in compact cluster; *pigment:* single dark mass.	 Immature pointed-end forms uncommon. *Mature forms:* banana-shaped or rounded; *chromatin:* single, well defined; *pigment:* scattered, coarse, rice-grain like; pink extrusion body sometimes present. Eroded forms with only chromatin and pigment often seen.
P. vivax	All stages seen; Schüffner's stippling in 'ghost' of host red cells, especially at film edge.	 *Size:* small to large; *number:* few to moderate; *shape:* broken ring to irregular forms common; *chromatin:* single, occasionally two; *cytoplasm:* irregular or fragmented; *mature forms:* compact, dense; *pigment:* scattered, fine.	 *Size:* large; *number:* few to moderate; *mature forms:* 12–24 merozoites, usually 16, in irregular cluster; *pigment:* loose mass.	 Immature forms difficult to distinguish from mature trophozoites. *Mature forms:* round, large; *chromatin:* single, well defined; *pigment:* scattered, fine. Eroded forms with scanty or no cytoplasm and only chromatin and pigment present.
P. ovale	All stages seen; prominent Schüffner's stippling in 'ghost' of host red cells, especially at film edge.	 *Size:* may be smaller than *P. vivax*; *number:* usually few; *shape:* ring to rounded, compact forms; *chromatin:* single, prominent; *cytoplasm:* fairly regular, fleshy; *pigment:* scattered, coarse.	 *Size:* rather like *P. malariae*; *number:* few; *mature forms:* 4–12 merozoites, usually 8, in loose cluster; *pigment:* concentrated mass.	 Immature forms difficult to distinguish from mature trophozoites. *Mature forms:* round, may be smaller than *P. vivax*; *chromatin:* single, well defined; *pigment:* scattered, coarse. Eroded forms with only chromatin and pigment present.
P. malariae	All stages seen.	 *Size:* small; *number:* usually few; *shape:* ring to rounded, compact forms; *chromatin:* single, large; *cytoplasm:* regular, dense; *pigment:* scattered, abundant, with yellow tinge in older forms.	 *Size:* small, compact; *number:* usually few; *mature forms:* 6–12 merozoites, usually 8, in loose cluster; some apparently without cytoplasm; *pigment:* concentrated.	 Immature and certain mature forms difficult to distinguish from mature trophozoites. *Mature forms:* round, compact; *chromatin:* single, well defined; *pigment:* scattered, coarse, may be peripherally distributed. Eroded forms with only chromatin and pigment present.

After WHO Bench Aids for the identification of malaria, 3rd edition

Quality Assuring GMM Activities using a

Routine Internal Competence Assessment SOP

SOP – GMM – QA – ICA - 1

Quality Assurance of GMM Activities and Routine Internal Competence Assessment (ICA)

Preamble: The Background to: SOP-GMM-QA-1-(ICA)

For some years programme managers assumed that supervision of GMM-SOP based diagnosis in MEP' globally, was effective and satisfactory and that QA systems sensitively monitored these routines. This was found not to be the case and it was confirmed over time that the supervision and monitoring of GMM SOP methods, with some exceptions, left much room for improvement. This is a continuing global problem in control and elimination programmes worldwide.

Oman was largely an exception to this finding. The NMEP QA system has functioned well for many years, fully justifying designation of the Central Malaria Reference Laboratory, Muscat as a recognized WHO Malaria Laboratory of Excellence. Since 2006 it has been a regional focal point for GMM training and QA and it is based on many of these experiences that the following SOP – GMM – QA – ICA -1 is presented.

Evaluating the SOPs

Remember: The SOP being evaluated is a mirror of the SOP that you will be using for the evaluation. Any deviation from this is no longer an exercise in what the staff member should be doing, but maybe of what you might want them to be doing.

A good rule of thumb is: 'If you are evaluating something that the staff member, or process, has not been taught (to that person) as an SOP then you should not be evaluating performance in that way.

In effect, much evaluation (so-called QA/QC) is carried out by senior staff who have not themselves received the appropriate training in that activity.

The Oman NMEP QA of examined slides has been confirmed for many years. Monthly submissions are made, of all slides identified as positive and 10 % of negative slides from lower level laboratories to the next level up. The process has been more confirmatory than that of blinded re-examination but accuracy of diagnosis is very high among all levels of Microscopist and the system works well. Re-examination of slides is carried out by supervisor Microscopist's at each level, culminating at the Central Malaria Reference Laboratory where six qualified staff, each with a minimum ten years service and GMM experience, are in close touch with activities. Following NMEP policy each of these staff

also has a broad experience in Instructional Skills, leadership and laboratory management.Similarly, the NMEP is well aware that the re-examination of a percentage of blood films cannot fully identify areas where staff are unable, or incompletely, carrying out their duties and that these require the additional assessment of provided services such as Building Maintenance, Laboratory Infra-structure, Technical and Management skills, Leadership, Training and etc. In fact, this area is the one that must be evaluated on a longitudinal basis, in any programme, before the programme is able to meaningfully utilise any kind of external competency assessment (ECA).

The WHO manual on Microscopy Quality Assurance and the ECA SOP are both currently undergoing extensive evaluation in the search for consensus and we await final word on this ongoing question.

It is, however, relevant to point out here that the Oman NMEP has run a series of six-monthly (ICA) supervisory visits to selected laboratories for some time and that this has highlighted a number of solutions to identified problems.

As part of the continuing development of the ICA for all laboratories and staff, it is proposed that this SOP contains some minor changes that bring the evaluation system in line with what is internally appropriate - to sustain efficiency.

For convenience SOP - GMM - QA - ICA - 1 has been divided in to two parts as follows:

- The **Quality Assurance of examined blood films: SOP – GMM – QA – ICA – 1A**
- **Internal Assessment of the diagnostic services of the NMEP: SOP-GMM-QA-ICA-1B**

Although ICA – 1B is inextricably linked to ICA -1A, it is clear that only in the most exceptional situations can, or do, these two activities take place together. If ICA-1A (cross-checking) is seen as constantly ongoing, so too is ICA – 1B, albeit less obviously so, and frequently conducted by different evaluators from different disciplines. This is proposed to take place in a situation where numerous visits are made to functioning laboratories by different supervisory staff but seldom is an evaluation report submitted following the visit.

(A). The Quality Assurance of examined blood films:

SOP – GMM – QA – ICA – 1A

This monitoring system has functioned well for a long time and requires little rearranging but possibly some further, simple, bench-based refresher training.

In effect, this is the current, well-established, crosschecking of all positive, and 10% of all negative slides. These are referred monthly by each level of laboratory to the next highest level and cross-checked by a designated 'more senior' Microscopist in each situation. What is not allowed for at present is the circulation of suitable 'spot slides' of currently interesting GMM-based material to maintain learning and diagnostic standards at a high level.

Allowing for the distance between some of the more remote laboratories and their next level establishment, the timeline to meet SOP requirements should be, by end of:

Step 1:	Selection of all positive slides and 10% random negatives for despatch to next level for QA.
Step 2	Re-examination of submitted slides following the examination protocol.
Step 3:	In the appropriate record forms, collation and dispersal of the results of the cross-checking.
Step 4:	Further action as indicated by results from the QA such as, Bench-based retraining (short). Or longer re-training requiring time at next level up.

Errors of a more serious nature, such as the gross misidentification of parasites (major discrepancies), and which may require a longer period, of one or two days of intensive refresher training to correct, can be arranged as and where applicable with the microscopist being quickly corrected, brought back to standard and working again under some supervision.

Based on current experience, the workload of slides to examine is not excessive and is manageable, although by the time positive slides reach the Central Reference Laboratory they will have been examined and re-examined (confirmed) three or four times. In spite of this, or because of it, this is a highly sensitive and efficient routine.

(B). Internal Assessment of the Diagnostic and Support Services of the NMEP:

SOP-GMM-QA-ICA-1B

The objective of ICA-1B is that this sub-portion of the SOP must enquire into the wide range of all aspects of the malaria diagnostics laboratory service. Structural issues, such as laboratory design, health and safety; the regularity of supplies, storage conditions and maintenance of equipment, are all part of what must be evaluated, if only occasionally. While the general management of the laboratory and working relationships between different levels of staff are also essential to the smooth functioning of these particular levels of the service.

Thus the structural and building maintenance aspects may need attention to the one time repair of a leaking roof over the malaria bench, or the lack of an uninterrupted power supply to the microscope lamp -- and which must be reported to someone outside of the malaria diagnostics service but which clearly will affect the outcome of the daily GMM routine.

These issues are clearly and frequently made more complicated by the range of personnel responsible for resolving such problems and the 'red-tape' that exists.

Check lists to a certain extent deal with these questions and some WHO publications go extensively into this. However, a checklist must be selected and constructed carefully to ensure that the correct areas are being addressed and evaluated. Also, once a checklist is completed and forwarded to the next higher authority as part of a larger report, it is frequently forgotten and the 'leaking roof', for example, continues to leak to the increasing frustration and poor performance of workers on the spot.

These are problems largely of poor awareness and poor communication:

Poor awareness, that the 'lack of a continual power supply to the microscope' will mean the slide cannot be examined, according to the SOP, and which may even affect the patient's wellbeing.

Poor communication, where the laboratory manager, for example, is unaware that the OPD nurse is too busy to send EDTA treated blood samples to the laboratory quickly for processing and that morphological changes can then rapidly take place to parasites that result in a misdiagnosis. How does the laboratory manager solve this problem, and who with?

A suitably designed Visitor's book situated in each laboratory with the laboratory manager will go a long way to addressing some of these problems. A self-appraisal of identified conditions can be done by each Microscopist, who highlights what is adversely affecting their work, and submitted as an integral part of their regular reports.

In some programmes a Microscopist's Individual Logbook is issued to each Microscopist on GMM graduation, or qualification. As shown overleaf, this acts as a record of their daily work activities such as: slides examined, positive and negative slides seen, any supervision they may carry out, and training that may take place, and identifies other areas that may be in need of attention. It is an excellent record to assist with competence assessment evaluation and should follow each individual through their career – including supervisors and core personnel.

Personal Logbook – Cover page

Name:……Date log started: ….....

Place of work: ……..…….……..…….……....

Job title: …….……..… GMM Grade level: ……

Grade level awarded on ……... …….....

Notes:

1. This logbook is designed for you to keep a record of your daily job activities easily.
2. It is an important component for your work as a supervisor and a skilled malaria microscopist.
3. It will help you to report the work you do and in planning your work programme.
4. It helps you to keep a record of the number of blood films that you personally examine …
5. and those that you validate and 'cross-check from the staff that you supervise.
6. You will briefly record here any training activities that you may carry out, or are involved in.
7. It is also important to record here any further training that you may undergo.
8. The entries need to be verified by your supervisor on a regular basis and there is space to do that.

Please do not use this log-book for any other purpose and do not remove pages from it or alter entries in any way.

Contacts: …… Mobile: ……

email …… …… …… ……

If you have any suggestions that you think may help to improve the design of this logbook please inform your supervisor.

Please note: This page and the following page follow a landscape format.

Date:	Slides examined	Slides positive	P. Falciparum	P. vivax	Others
Your own slides:					
Source 1:					
Source 2					
Source 3					
Total for the day					
Supervisory visit to:		Brief comments:			
Supervisory visit or activity:		Brief comments			
Training carried out:					
Training undergone:					
Other comments:					

Date:	Slides examined	Slides positive	P. Falciparum	P. vivax	Others
Your own slides:					
Source 1:					
Source 2					
Source 3					
Total for the day					
Supervisory visit to:		Brief comments:			
Supervisory visit or activity:		Brief comments			
Training carried out:					
Training undergone:					
Other comments:					

Date:	Slides examined	Slides positive	P. Falciparum	P. vivax	Others
Your own slides:					
Source 1:					
Source 2					
Source 3					
Total for the day					
Supervisory visit to:		Brief comments:			
Supervisory visit or activity:		Brief comments			
Training carried out:					
Training undergone:					
Other comments:					

This page follows a landscape format to allow for more space in each box.

Supervisor's signature: …….Date: …….

Supervisor's signature: …….Date: …….

Annex

GMM – Standard Operating Procedures, National Malaria Eradication Programme

SOP title: (Draft copy for clearance)	SOP number:

	Name(s)	Title(s)	Signature(s)	Date
Author(s)				
Reviewers				
Authority				

Effective date:	
Review dates:	

Read and understood by USER:			
Name	Title of Post	Signature, read and understood	Date

See instructions next page.

Reference materials for this series of SOPs and QA exercises:

1. **Basic Malaria Microscopy, Parts 1 and 2, WHO Geneva 2010; The Tutor's Guide to be made available for trainers while one copy each of the Learner's Guide to be available on the work bench.**

2. **Bench Aids for the diagnosis of malaria infections, 3rd Edition, WHO Geneva, 2009; one set per participant.**

3. **Malaria Microscopy Quality Assurance Manual, Version 1, WHO Geneva 2009. For Reference purposes available to Core SOP Group.**