The MalariaCare Toolkit

Tools for maintaining high-quality malaria case management services

MalariaCare diagnostics refresher training learner’s manual

Download all the MalariaCare tools from: www.malariacare.org/resources/toolkit.
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## Abbreviations

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>$P.$</td>
<td><em>Plasmodium</em></td>
</tr>
<tr>
<td>$p$LDH</td>
<td><em>Plasmodium</em> lactate dehydrogenase</td>
</tr>
<tr>
<td>RDT</td>
<td>rapid diagnostic test</td>
</tr>
<tr>
<td>RBC</td>
<td>red blood cell</td>
</tr>
<tr>
<td>SOP</td>
<td>standard operating procedure</td>
</tr>
</tbody>
</table>
Background

As part of its mandate to improve the quality of malaria case management, including diagnosis and treatment, MalariaCare has developed a series of short courses aimed at training providers to improve skills to diagnose and treat malaria and other febrile cases. This document provides guidelines for conducting malaria diagnosis refresher training in a classroom setting. However, this document or parts thereof may be useful for trainers, mentors, and supervisors when reinforcing provider skills in diagnosis of malaria during supervision and mentorship activities.

Course objectives

The objective of the course is to enable the participants to acquire and develop essential knowledge and competencies in malaria diagnosis (see Annex 1: Sample timetable) and quality assurance schemes. On completion of the course, the participants should be able to:

• Demonstrate an understanding of the epidemiology of malaria.
• Describe the biology of the malaria vector and parasite.
• Prepare high-quality stains for malaria diagnosis.
• Prepare thick and thin blood films and stain films to a high standard.
• Identify all malaria species (*Plasmodium falciparum, P. vivax, P. ovale, P. malariae*) microscopically.
• Identify all malaria parasite stages microscopically.
• Differentiate artifacts and pseudoparasites from true malaria parasites and identify other blood parasites.
• Quantify malaria parasites accurately using a counting method.
• Carry out malaria rapid diagnostic tests (RDTs) correctly.
• Monitor the performance of malaria RDTs.
• Participate in developing national and facility-based plans for quality assurance in malaria diagnosis.
• Develop and maintain standard operating procedures (SOPs).
• Perform technical work according to standards of good laboratory practice.
• Participate in development of national plans for regular support supervision and onsite training and mentoring.

Number and selection of participants

Selection of participants

Laboratory technologists/technicians from the national level will be called to attend this workshop. The identified staff should be working mainly in the malaria/parasitology section of the laboratory. They will be expected to take a lead in establishing the relevant laboratory procedures, sharing their updated knowledge with the other staff in their health facilities, and supervising staff in their own health facilities and facilities under their supervision.
Venue selection and length of course

An appropriate venue should be identified with the following specifications:

- A venue with a room for teaching and laboratory work, or separate rooms for teaching and laboratory work.
- One functioning electric binocular microscope per participant.
- Mains electricity and/or access to a power generator.
- A facility to provide morning and afternoon tea and lunch to the participants.
- Transport to collect additional supplies, and secretarial assistance. Access to printing and photocopying is an added advantage.
- Facilities for accommodation of facilitators and participants nearby.

Duration of training

The duration of the training is five days.

Course evaluation

This course utilizes a 360-degree approach to assessment, meaning the course participants are evaluated as well as the trainers/facilitators.

Evaluation of course participants is carried out in the following ways:

- Pre-tests and post-tests (theory and practical).
- Observation by facilitators during the course.
- Competency assessment of malaria microscopy.

Grading course participants

Malaria microscopy

Pass marks in malaria microscopy performance for core supervisors are based on the World Health Organization minimum scores for reference-level microscopists scoring at level 2 for parasite detection and quantitation:

- >80% parasite detection.
- >40% density.

For those participants that are planning to attend a World Health Organization External Competency Assessment in the near future, the following scores are recommended:

- >80% parasite detection.
- >80% species identification.
- >40% density.
**Rapid diagnostic tests**

Pass marks for RDT competence is >85% (based on observation checklist).

**Participants' evaluation of the course**

At the end of the course, the participants will be asked to fill out a standard evaluation form. The evaluation will be conducted anonymously and will address both technical and logistical issues.

**Evaluation of the impact of the course**

This will be by regular (ideally quarterly) review of individuals:

- During routine and regular proficiency testing using the slide bank (consisting of 300 slides).

**Required supplies, equipment, and materials**

Supplies and equipment should be organized well in advance of the actual training date. Electric microscope quality needs to be inspected by trainers to ensure that the necessary components are available (i.e., 100X immersion oil objective, lead, etc.). Extra bulbs should be on hand in order to respond to issues on the spot.
DAY ONE

Introduction to the course (1 hour)

The learning objectives of this unit are to introduce the course content, set the ground rules for the course, and perform the pre-tests.

<table>
<thead>
<tr>
<th>Objectives: Introduction to the course</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Request the participants to introduce one another.</td>
</tr>
<tr>
<td>• Explain the objectives of the refresher training course.</td>
</tr>
<tr>
<td>• Establish the expectations of the participants.</td>
</tr>
<tr>
<td>• Review the timetable for the course.</td>
</tr>
<tr>
<td>• Stipulate the ground rules for the course and review administrative issues.</td>
</tr>
<tr>
<td>• Establish the governance for the course duration.</td>
</tr>
</tbody>
</table>

Content

• **Introduction**: name, length of time in service, place of work, designation at work.

• **Objectives of the refresher training course**: understanding the role of the laboratory in diagnosis, control, and prevention of diseases; understanding the principles of laboratory tests; improving technical skills in laboratory practice; and selecting, using, and caring for laboratory equipment.

• **Expectations of the course**: improved malaria diagnosis and case management of patients, and clearly defined role in quality assurance activities.

• **Timetable for the course**: comments on timetable, suggested changes.

• **Setting ground rules**: importance and purpose of ground rules, selecting ground rules, following ground rules throughout the course.

• **Establishing the governance of the course**: administrative structures, team leader, timekeeper, rapporteurs.

Notes:
Pre-assessment—theory, malaria microscopy, and RDTs (4 hours)

The pre-test is used to measure existing knowledge and competencies of laboratory staff with respect to microscopic diagnosis of malaria.

<table>
<thead>
<tr>
<th>Learning objectives: Pre-assessment—theory, malaria microscopy, and RDTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Identify areas of technical proficiency and laboratory management in malaria diagnosis.</td>
</tr>
<tr>
<td>• Determine the competency of individual participants in malaria parasite detection, species identification, and parasite quantification.</td>
</tr>
<tr>
<td>• Determine the competency of individual participants in malaria rapid diagnostic tests.</td>
</tr>
<tr>
<td>• Inform each participant of their performance.</td>
</tr>
</tbody>
</table>

Content

- **Pre-tests:** purpose of pre-tests, format of laboratory theory tests, format of practical test.
- **Determining the competency of individual participants:** detection of malaria parasites, species identification, and accuracy of parasite quantification.
- **Determine the competency of individual participants in malaria rapid diagnostic tests:** based on an observation checklist.
- **Informing each participant** of their respective scores on pre- and post-tests.

Notes:
Learning Unit 1

Malaria situation and policies (1 hour)

Review the epidemiology of malaria and review strategies and progress made on malaria control.

<table>
<thead>
<tr>
<th>Learning objectives: Malaria situation and policies</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Describe the epidemiology of malaria.</td>
</tr>
<tr>
<td>• Recognize and understand the vectors of malaria.</td>
</tr>
<tr>
<td>• Articulate the progress made in malaria control.</td>
</tr>
<tr>
<td>• Understand the policies governing microscopy and RDTs.</td>
</tr>
</tbody>
</table>

Content

- **Epidemiology of malaria:** factors affecting distribution of malaria, climate, and geographical features.
- **Review the diagnostic and treatment policies/algorithms for uncomplicated and severe malaria.**
- **Malaria vectors:** mosquito species, vector behavior, epidemiology of vectors in the country.
- **Progress made in malaria control:** control strategies, implementation of malaria control strategies, monitoring of outcomes.

Notes:

END OF DAY ONE
DAY TWO

Learning Unit 2

Life cycle of malaria parasites, parasite morphology, clinical symptoms in relation to the parasite life cycle (1 hour)

The aim of this learning unit is to equip participants with knowledge of the malaria life cycle and its importance in the presentation and diagnosis of malaria.

<table>
<thead>
<tr>
<th>Learning objectives: Parasite life cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Describe the life cycle of malaria parasites in man and the vector.</td>
</tr>
<tr>
<td>• Define specific terms and parasite names in relation to the malaria life cycle.</td>
</tr>
<tr>
<td>• Describe exoerythrocytic schizogony and the pre-patent and incubation periods of the four malaria parasite species.</td>
</tr>
<tr>
<td>• Describe the common symptoms of uncomplicated and severe malaria in relation to the parasite life cycle.</td>
</tr>
<tr>
<td>• Describe development and spread of drug resistance.</td>
</tr>
</tbody>
</table>

Content

• **Describe the life cycle of malaria parasites in man and the vector:** life cycle in man, life cycle in the vector, stages of malaria parasite development, stages in man in relation to clinical symptoms, stages in man in relation to parasite morphology, stages in the vector.

• **Definition of terms and parasite names:** recrudescence, relapse, hypnozoite, names of four species, and stages.

• **Morphological features of malaria parasite species at different stages of the life cycle:** trophozoites, schizonts, gametocytes of *P. falciparum*, *P. malariae*, *P. vivax*, and *P. ovale*.

• **Common symptoms of uncomplicated and severe malaria:** key diagnostic features of uncomplicated and severe malaria in children, adults, pregnant women, and immune and nonimmune individuals; laboratory confirmation versus use of clinical symptoms and signs for diagnosis.

Notes:
Learning Unit 3

Parasitological stains: Preparation of Giemsa (1 hour)

The aim of this learning unit is to update participants on a systematic approach to staining blood films for malaria parasites.

<table>
<thead>
<tr>
<th>Learning objectives: Preparation of Giemsa stain</th>
</tr>
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<tbody>
<tr>
<td>• Principles of Romanowsky stains.</td>
</tr>
<tr>
<td>• Equipment and reagents for stain preparation.</td>
</tr>
<tr>
<td>• Demonstration of correct operation of weighing scale.</td>
</tr>
<tr>
<td>• Preparation of stains.</td>
</tr>
<tr>
<td>• Preparation of buffer for diluting Giemsa stain.</td>
</tr>
<tr>
<td>• Prevention of artifacts and contaminants.</td>
</tr>
<tr>
<td>• Quality control of stains.</td>
</tr>
</tbody>
</table>

Content

• **Principles of Romanowsky stains**: concepts of basic/acidic components of the stain and cells, azures of methylene blue, eosin, methanol-based stains, water-based stains.

• **Equipment and reagents for stain preparation**: weighing scales, pH meter, glassware, reagent bottles, Giemsa powder, glycerol, methanol, buffers.

• **Weighing scale operation**: ensuring cleanliness of the balance, setting the balance, weighing in a wind-free environment, using weights, using filter paper to protect the pan.

• **Preparing stains**: weighing powders, mixing powders, using distilled water or rainwater, labeling correctly.

• **Preparing buffer**: weighing buffer powders, measuring distilled water, mixing, adjusting pH.

• **Prevention of artifacts, contaminants**: filtering stains, covering stains, discarding diluted Giemsa after six hours.

• **Quality control of stains**: testing new batches of stain against known positive and negative films, quality control of stock stains.

Notes:
Learning Unit 4

Collection of capillary and venous blood (1 hour)

The aim of this learning unit is to provide refresher training on blood collection techniques for malaria diagnosis.

<table>
<thead>
<tr>
<th>Learning objectives: Collection of capillary and venous blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Effects of different anticoagulants on parasitized red blood cells.</td>
</tr>
<tr>
<td>• Discuss and perform collection of capillary and venous blood samples and use of anticoagulants.</td>
</tr>
</tbody>
</table>

Content

- Effects of anticoagulants on parasitized red blood cells (RBCs): changes in morphology of RBCs, selection of anticoagulants.
- Collecting blood samples: capillary blood, venous blood, use of different anticoagulants.

Notes:
Learning Unit 5

Preparation and staining of thick and thin blood films (1 hour)

The aim of this learning unit is to update participants on a systematic approach to preparing blood films for malaria parasite examination.

Learning objectives: Preparation and staining of thick and thin blood films

- Preparation of thick blood films.
- Preparation of thin blood films.
- Staining thick blood films.
- Fixing and staining thin blood films.
- Prevention of artifacts.

Content

- **Preparing thick blood films**: using cleaned slides, identifying patient, spreading in a circle, size and thickness of film, using a template, drying away from artificial heat or sunlight.
- **Preparing thin blood films**: using cleaned slides, identifying patient, selecting spreader, spreading films, spreading anemic blood, using a template, drying away from artificial heat or sunlight.
- **Staining thick blood films**: preparing buffered solutions, diluting Giemsa stain, staining with Giemsa stain.
- **Fixing and staining thin blood films**: preparing buffered solutions, diluting Giemsa stain, fixing thin films with methanol, staining with Giemsa stain.
- **Prevention of artifacts**: cleaning slides, drying in a protected area, avoiding use of artificial drying.

Microscopic assessment (3 hours)

Participants will read a set of 15 slides each day and report on parasite detection, species identification, and parasite density.

END OF DAY TWO

Notes:
DAY THREE

Review of slides (30 minutes)

Each day, participants will have an opportunity to review slides from the previous day’s assessment.

Learning Unit 6

Examination and reporting of blood films (1 hour)

To update participants on a systematic approach to malaria microscopy, species identification, and parasite quantification.

Learning objectives: Examination and reporting of blood films

- Discuss laboratory diagnosis of malaria using light microscopy, advantages and disadvantages of malaria microscopy.
- Describe and identify normal cellular components of blood.
- Recognize and identify different species and stages of malaria parasites and mixed infections.
- Explain the purpose of malaria parasite quantification.
- Describe quantification methods on thick and thin blood films.
- Perform quantification methods on thick and thin blood films.
- Describe and demonstrate the standard reporting format.
- Report malaria parasite quantification.

Content

- **Laboratory diagnosis of malaria using light microscopy**: advantages and disadvantages—of microscopy as the accepted standard method for detecting malaria parasites in blood; requirements for trained laboratory personnel and equipment (functional microscope); other logistical requirements.
- **Normal components of blood**: white blood cell, RBC, platelets,
- **Identifying different species and stages of malaria parasites**: morphology of different stages of *Plasmodium* species: *P. falciparum, P. malariae, P. ovale, P. vivax*, trophozoites, schizonts, gametocytes, mixed infections.
- **Purpose of malaria parasite quantification**: measuring parasite density, assessing response to treatment.
- **Quantification method on thick blood films**: technique of counting on a thick film, interpretation.
- **Quantification methods on thin blood films**: technique of counting on a thin film, interpretation.
- **Reporting format for malaria parasites**: positive and negative, parasite species, developmental stages, density (parasites/microliter).
- **Reporting malaria parasite quantification**: thick films, thin films, counting parasites per white blood cells, counting parasitized RBC against total RBCs, calculating parasites per microliter, calculating percent parasitemia.
Learning Unit 7

Artifacts, pseudoparasites, mixed infection, other blood parasites (30 minutes)

To update participants on a systematic approach to recognizing common artifacts, pseudoparasites, mixed infections, and other blood parasites in blood films.

Learning objectives: Artifacts, pseudoparasites, mixed infection, other blood parasites

• Recognize common artifacts.
• Recognize pseudoparasites.
• Recognize and identify other blood parasites.

Content

• Recognition of artifacts: stain deposits, dust, salts, scratches on slides.
• Recognition of pseudoparasites: fungi, bacteria.
• Recognition of mixed infections: thick and thin blood films.
• Recognition of other blood parasites: Borrelia species, trypanosomes, microfilariae, differences from malaria parasites.

Notes:
Learning Unit 8

Malaria rapid diagnostic tests (30 minutes)

To update participants on a systematic approach to using and monitoring malaria RDTs.

**Learning objectives: Rapid diagnostic tests**

- Principle of malaria RDTs.
- Procedures for using malaria RDTs.
- Advantages and disadvantages of using RDTs.
- Common user errors and implications for test results.
- Storage of malaria RDTs.
- Quality assurance for malaria RDTs.

**Content**

- **Principles of RDTs:** HRP-2-based tests, *Plasmodium* lactate dehydrogenase (pLDH) and aldolase-based tests, types of devices.
- **Procedures for using malaria RDTs:** collection of blood sample, test procedure, test interpretation, use of job aids.
- **Advantages and disadvantages of RDTs:** sensitivity, species identification, need for quantification, utilization, stability of proteins, storage requirements, cost, equipment, staff training.
- **Common user errors:** use of expired tests, not allowing test kits to warm to room temperature, correct dispensing of reagents fluids, accurate timing, correct reading of test and control lines, clerical errors.
- **Storage of malaria RDTs:** effects of temperature and humidity, storage methods, monitoring of storage conditions.
- **Quality control procedures:** checking expiry dates, checking storage temperatures, checking packaging integrity, checking against blood slides.
- **Quality assurance of malaria RDTs:** pre-qualification testing, lot testing, field-testing methods.

**Notes:**
Learning Unit 9

Practical session for preparation and staining of a thick and thin blood film (2 hours)

To provide participants with an opportunity to both practice and receive feedback on making a thick and thin blood film.

<table>
<thead>
<tr>
<th>Learning objectives: Practical session for preparation and staining of a thick and thin blood film</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Prepare stain.</td>
</tr>
<tr>
<td>• Collect venous blood.</td>
</tr>
<tr>
<td>• Prepare thick and thin blood film.</td>
</tr>
</tbody>
</table>

Microscopic assessment (3 hours)

Participants will read a set of 15 slides each day and report on parasite detection, species identification, and parasite density.

END OF DAY THREE

Notes:
DAY FOUR

Review of slides/RDT post-test (2 hours)

Each individual will be observed performing an RDT against a standard observation checklist for RDTs. The class will have an opportunity to review misread slides from the week while waiting for their turn.

Learning Unit 10

Malaria diagnostics quality assurance (1 hour)

To update participants on malaria diagnostic quality assurance activities.

<table>
<thead>
<tr>
<th>Learning objectives: Malaria diagnostics quality assurance</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Definitions of quality.</td>
</tr>
<tr>
<td>• Laboratory errors.</td>
</tr>
<tr>
<td>• External quality assessment schemes (current and planned).</td>
</tr>
<tr>
<td>• Internal quality control for health facilities.</td>
</tr>
<tr>
<td>• Role of the national reference laboratory and core group.</td>
</tr>
<tr>
<td>• Monitoring indicators of competency for core group.</td>
</tr>
</tbody>
</table>

Content

- **Definitions of quality**: quality assurance, internal quality control, external quality assessment.
- **Laboratory errors**: causes of errors, types of errors.
- **Quality assurance in practice**: patient replicates, cumulative sum (cusum) charts.
- **External Quality Assessment Schemes**: methods of conducting External Quality Assessment Schemes, proficiency testing, annual competency assessment.
- **Internal quality control for health facilities**: positive and negative controls, SOPs, test validation.
- **Role of the national reference laboratory and core group**: policy development, refresher training, supervisory systems, slide rechecking.
- **Indicators of competency for core group**: minimum standards for malaria microscopy and RDTs for the national reference group, knowledge of national diagnostic and treatment policies.

Notes:
Learning Unit 11

Overview of supervisory visit (2 hours)
To ensure participants understand the flow and content of the supervisory visit.

<table>
<thead>
<tr>
<th>Learning objectives: Overview of supervisory visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Overview of the visit and integrated checklist (malaria components).</td>
</tr>
<tr>
<td>• The role of the reference group in supervision.</td>
</tr>
<tr>
<td>• Role-playing use of the supervision checklist.</td>
</tr>
<tr>
<td>• Identify best practices for malaria diagnosis.</td>
</tr>
<tr>
<td>• Debrief health facility staff and authorities on visit conclusions and recommendations, continuous quality improvement.</td>
</tr>
<tr>
<td>• Summarize key points to be made when briefing local administrators on promoting program support.</td>
</tr>
</tbody>
</table>

Content
• Overview of the visit: frequency, length of time spent at health facility, expectation for the visit, supervisor and health facility staff.
• Review the integrated supervision checklist (malaria component) and clarify each question for common understanding.
• How to appropriately fill in the supervision checklist.
• The role of the reference group in supervision: slide rechecking, onsite training, observation, mentoring, and corrective actions.
• Role-playing use of the supervision checklist: flow and comprehension.
• Groups provide feedback on role-playing experience and identify best practices for malaria diagnosis: internal quality control, quality assurance, SOPs.
• Debriefing appropriate staff and authorities on visit conclusions, recommendations, and how to utilize previous visit data for continuous quality improvement.

Microscopic assessment (3 hours)
Participants will read a set of 15 slides each day and report on parasite detection, species identification, and parasite density.

END OF DAY FOUR

Notes:
DAY FIVE

Learning Unit 12

Sources of error (1 hour)

Review common sources of errors that occur throughout the diagnostic cycle.

<table>
<thead>
<tr>
<th>Learning objectives: Sources of error</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Understand the clinical and laboratory diagnostic cycle.</td>
</tr>
<tr>
<td>• Define pre-analytical, analytical, and post-analytical phases of the diagnostic process.</td>
</tr>
<tr>
<td>• Identify common errors that occur in each of the pre-analytical, analytical, and post-analytical phases in places of work.</td>
</tr>
<tr>
<td>• Suggest practical solutions to the errors in each phase.</td>
</tr>
</tbody>
</table>

Content

• **Understand the clinical and laboratory diagnostic cycle**: roles of clinical and laboratory staff, flow of patients between the clinic and laboratory, importance of a managed approach to every step in the cycle.

• **Define pre-analytical, analytical and post-analytical phases of diagnosis**: understanding the steps in each phase, understanding laboratory as well as clinical processes.

• **Identify common errors that may occur in each of the pre-analytical, analytical, and post-analytical phases in places of work**: errors due to clinicians, errors due to laboratory staff, errors due to support staff, errors due to lack of resources, errors due to poor reporting, errors due to delays.

• **Suggest practical solutions to the errors in each phase**: solutions that cost nothing (reorganization of patient flow, instructions to patients, proper labeling, correct laboratory procedures); solutions that cost very little (laboratory request forms, correct laboratory reagents); solutions that cost more (new equipment, more staff).

Notes:
Learning Unit 13

Laboratory safety for malaria diagnostics (1 hour)

The aim of this session is to introduce participants to general laboratory safety and precautions against laboratory-acquired infections.

Learning objectives: Laboratory safety for malaria diagnostics

- Define general laboratory safety.
- Universal precautions for preventing laboratory-acquired infections.
- Describe safe waste management procedures for microscopy and RDTs.

Content

- **General laboratory safety**: work environment, safety equipment, types of hazards, disinfection, and sterilization.
- **Universal precaution**: use of protective barriers such as gloves, gowns, aprons, safety practices, no mouth pipetting.
- **Safety in waste disposal**: disposal of stains, sharps, RDTs and other related chemicals, reagents.

Notes:
Learning Unit 14

Develop quality improvement work plans (1 hour)

The aim of this session is to devise a plan to disseminate information from this workshop to staff in the participants’ health facilities and the health facilities under their supervision.

<table>
<thead>
<tr>
<th>Learning objectives: Develop quality improvement work plans</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Take a lead in establishing the relevant laboratory procedures in places of work.</td>
</tr>
<tr>
<td>• Devise a work plan to share the knowledge with the other staff in their health facilities, and improve the quality of diagnostic services in their facilities/counties.</td>
</tr>
<tr>
<td>• Develop a list of health facilities that they supervise.</td>
</tr>
<tr>
<td>• Develop list of essential equipment and supplies needed to conduct a supervisory visits.</td>
</tr>
</tbody>
</table>

Content

- **Participants taking a lead in establishing the relevant laboratory procedures**: making plans to create SOPs in the participants’ health facilities in line with national and international guidelines.
- **Devise work plans to share knowledge with the other staff in health facilities**: schedule time to review information learned at this workshop with members of each participants’ health facility, training of others.

Learning Unit 15

Presentation of individual work plans

Individuals will share individual work plans for targeted quality improvement for their own health facility.

Learning Unit 16

Presentation of final microscopy and RDT competency results

Post-test results on malaria microscopy and RDT competency will be shared with the group.

END OF DAY FIVE
# Annexes

## Annex 1: Sample timetable for refresher training in laboratory diagnosis of malaria

<table>
<thead>
<tr>
<th>Time</th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30–9:00</td>
<td>Introduction: ground rules, expectations</td>
<td>Review pre-test slides</td>
<td>Review pre-test slides</td>
<td></td>
<td>Learning Unit 12: Sources of error</td>
</tr>
<tr>
<td>9:00–9:30</td>
<td></td>
<td>Learning Unit 2: Life cycle of malaria parasite</td>
<td>Learning Unit 6: Examination reporting of blood films</td>
<td></td>
<td>Learning Unit 13: Laboratory safety</td>
</tr>
<tr>
<td>9:30–10:00</td>
<td></td>
<td>Learning Unit 3: Parasitological stains (practical)</td>
<td></td>
<td>Review of slides RDT post-test</td>
<td></td>
</tr>
<tr>
<td>10:00–10:30</td>
<td></td>
<td>Learning Unit 4: Collection of capillary and venous blood (theory and practical)</td>
<td>Learning Unit 7: Artifacts, mixed infections, &amp; other parasites (theory)</td>
<td></td>
<td>Learning Unit 14: Developing quality improvement work plans</td>
</tr>
<tr>
<td>10:30–11:00</td>
<td>Pre-assessment: theory, malaria microscopy, and RDTs</td>
<td>Learning Unit 5: Prep &amp; staining of thick and thin blood films</td>
<td>Learning Unit 8: Malaria rapid diagnostic tests (theory)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00–11:30</td>
<td></td>
<td>Learning Unit 9: Practical session for preparation and staining of thick and thin blood films</td>
<td>Learning Unit 10: Malaria diagnostics quality assurance</td>
<td></td>
<td>Learning Unit 15: Presentation of individual work plans</td>
</tr>
<tr>
<td>11:30–12:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:00–12:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:30–1:30</td>
<td>Lunch</td>
<td>Lunch</td>
<td>Lunch</td>
<td>Lunch</td>
<td>Lunch</td>
</tr>
<tr>
<td>1:30–2:00</td>
<td>Pre-assessment: (continuation)</td>
<td>Learning Unit 5 (continuation)</td>
<td>Learning Unit 9 (continuation)</td>
<td>Learning Unit 11 (continuation)</td>
<td>Learning Unit 15: Group exercise</td>
</tr>
<tr>
<td>2:00–2:30</td>
<td></td>
<td>Daily Assessment Examination &amp; reporting blood films (practical)</td>
<td>Daily Assessment Examination &amp; reporting blood films</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2:30–3:00</td>
<td>Learning Unit 1: Malaria situation and policies</td>
<td>Microscopic Assessment</td>
<td></td>
<td>Microscopic Assessment</td>
<td>Learning Unit 16: Final competency results</td>
</tr>
<tr>
<td>3:00–3:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3:30–4:00</td>
<td>Coffee Break</td>
<td>Coffee Break</td>
<td>Coffee Break</td>
<td>Coffee Break</td>
<td>Coffee Break</td>
</tr>
<tr>
<td>4:00–4:30</td>
<td>Mentorship</td>
<td>Microscopic Assessment (continuation)</td>
<td>Microscopic Assessment (continuation)</td>
<td>Microscopic Assessment (continuation)</td>
<td>Learning Unit 16 (continuation)</td>
</tr>
<tr>
<td>4:30–5:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Closing remarks and departure</td>
</tr>
<tr>
<td>5:00–5:30</td>
<td></td>
<td>Rapid diagnostic tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6:00–7:00</td>
<td>Dinner</td>
<td>Dinner</td>
<td>Dinner</td>
<td>Dinner</td>
<td>Dinner</td>
</tr>
</tbody>
</table>
Annex 2: Pre- and post-tests

PRE-TEST: REFRESHER TRAINING FOR LABORATORY MALARIA DIAGNOSIS

Name: ____________________________
Date: ____________________________

Instructions: Circle the correct answers. Each question may have more than one correct answer. Please do not guess. A half a mark will be deducted for every wrong answer circled.

1. Quality assurance in the laboratory:
   a) Is best practiced by the laboratory in charge.
   b) Sample collection techniques are not important.
   c) Standard operating procedures (SOPs) are an important component.
   d) Equipment calibration is not a necessary prerequisite.

2. Quality assurance includes:
   a) Internal quality control.
   b) Process control.
   c) Disciplining errant members of staff.
   d) Detecting parasites in thick blood films.

3. Intra-laboratory proficiency testing involves:
   a) Comparison between technicians in a laboratory.
   b) Comparison between members from different laboratories.
   c) Closeness of repeated tests of the same quality.
   d) Exactness of a measured value compared to a true value.

4. What is the correct light adjustment when examining stained preparations using the 100X oil immersion objective?
   a) Lamp rheostat fully open, condenser lowered, iris diaphragm fully open.
   b) Lamp rheostat fully open, condenser raised, iris diaphragm fully open.
   c) Lamp rheostat fully open, condenser raised, iris diaphragm partially closed.
   d) Lamp rheostat fully open, condenser lowered, iris diaphragm partially closed.

5. Giemsa stain has the following characteristics:
   a) An alcohol-based Romanowsky stain.
   b) Can be used for staining thin blood films.
   c) Readily contaminated by bacteria and molds.
   d) Prepared by dissolving 5 percent of Giemsa powder.

6. Red blood cells (RBCs) infected with *Plasmodium malariae* are:
   a) Occasionally smaller in size than normal RBCs.
   b) Larger than normal RBCs.
   c) Fimbriated.
   d) Resemble RBCs infected with *P. vivax*.
7. The following are required to perform a malaria parasite count:
   a) A computer with a statistical program.
   b) Tally counters.
   c) Well-stained blood films.
   d) Lancets.

8. When preparing a thick blood film, the following are required:
   a) Timer.
   b) Spreader.
   c) Tally counters.
   d) Glass slides.

9. The following may be seen during malaria microscopy:
   a) Spirochaetes.
   b) LD bodies.
   c) *Onchocerca volvulus*.
   d) *Trypanosoma gambiense*.

10. Which of the following are true for field stain when used for staining blood films for malaria parasite examination?
    a) It is an alcohol-based Romanowsky stain.
    b) It stains the nucleus of parasites blue and the cytoplasm red.
    c) It can be used for staining thin blood films.
    d) One of the components is the azures of methylene blue.

11. The following diseases or conditions must always be confirmed in the laboratory in children above five years old:
    a) Malaria.
    b) Fungal infection of the skin.
    c) Madura foot.
    d) Anemia.

12. Crenated RBCs observed during malaria infection are commonly associated with:
    a) *P. malariae*.
    b) *P. ovale*.
    c) *P. falciparum*.
    d) *P. vivax*.

13. Rapid diagnostic tests for malaria:
    a) Detect HRP-2 antigens.
    b) Detect HRP-2 antibodies.
    c) Can differentiate *P. falciparum* from other species of malaria.
    d) Detect substances produced by trophozoites and young gametocytes.

14. SOPs in the laboratory are important for:
    a) Improving and maintaining quality of laboratory services.
    b) Informing the medical superintendent of laboratory results.
    c) Identifying problems associated with poor working performance.
    d) Improving specimen-collecting techniques.
15. Which of the following forms are necessary in a well-functioning laboratory?
   a) Laboratory request forms.
   b) Patient clinical forms.
   c) Laboratory report forms.
   d) ART requisition forms.

16. Laboratory data cannot be used for:
   a) Requesting additional staff.
   b) Quantifying the reagents needed.
   c) Giving an indicator of disease burden in the region.
   d) Patient counseling.

17. Collection of blood samples for blood parasites should involve:
   a) Collection of blood when the patient has fever.
   b) Blood film preparation as soon as possible.
   c) Taking the sample at any time.
   d) Patients do not need to be prepared.

18. Malarial anemia exists when:
   a) Hemoglobin level is reduced below the normal level for age, sex, and altitude.
   b) The bone marrow is suppressed.
   c) There is an increased destruction of parasitized and unparasitized cells.
   d) The blood cells appear hypochromic and microcytic.

19. Severe malarial anemia:
   a) Can be corrected by blood transfusion.
   b) May be associated with prostration.
   c) Is always fatal.
   d) Is associated with hemoglobin <5 g/dl.
## Annex 3: Comparison of *Plasmodium* species that cause malaria

### Comparison of the *Plasmodium* Species Which Cause Human Malaria

<table>
<thead>
<tr>
<th><em>Plasmodium</em> Species</th>
<th>Stages found in blood</th>
<th>Appearance of Erythrocyte (RBC)</th>
<th>Appearance of Parasite</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P. falciparum</strong></td>
<td>Ring</td>
<td>normal; multiple infection of RBC more common than in other species</td>
<td>delicate cytoplasm; 1-2 small chromatin dots; occasional applique (accolie) forms</td>
</tr>
<tr>
<td></td>
<td>Trophozoite</td>
<td>normal; rarely Maurer’s clefts (under certain staining conditions)</td>
<td>seldom seen in peripheral blood; compact cytoplasm; dark pigment</td>
</tr>
<tr>
<td></td>
<td>Schizonte</td>
<td>normal; rarely Maurer’s clefts (under certain staining conditions)</td>
<td>seldom seen in peripheral blood; mature = 8-24 small merozoites; dark pigment, clumped in one mass</td>
</tr>
<tr>
<td></td>
<td>Gametocyte</td>
<td>distorted by parasite</td>
<td>crescent or sausage shape; chromatin in a single mass (macrogametocyte) or diffuse (microgametocyte); dark pigment mass</td>
</tr>
<tr>
<td><strong>P. vivax</strong></td>
<td>Ring</td>
<td>normal to 1/4 X; round; occasionally fine Schuffner’s dots; multiple infection of RBC not uncommon</td>
<td>large cytoplasm with occasional pseudopods; large chromatin dot</td>
</tr>
<tr>
<td></td>
<td>Trophozoite</td>
<td>enlarged 1/2-2 X; may be distorted; fine Schuffner’s dots</td>
<td>large ameoboid cytoplasm; large chromatin; fine, yellowish-brown pigment</td>
</tr>
<tr>
<td></td>
<td>Schizonte</td>
<td>enlarged 1/2-2 X; may be distorted; fine Schuffner’s dots</td>
<td>large, may almost fill RBC; mature = 12-24 merozoites; yellowish-brown, coalesced pigment</td>
</tr>
<tr>
<td></td>
<td>Gametocyte</td>
<td>enlarged 1/2-2 X; may be distorted; fine Schuffner’s dots</td>
<td>round to oval; compact; may almost fill RBC; chromatin compact, eccentric (macrogametocyte) or diffuse (microgametocyte); scattered brown pigment</td>
</tr>
<tr>
<td><strong>P. ovale</strong></td>
<td>Ring</td>
<td>normal to 1/4 X; round to oval occasionally Schuffner’s dots; multiple infection of RBC not uncommon</td>
<td>sturdy cytoplasm; large chromatin</td>
</tr>
<tr>
<td></td>
<td>Trophozoite</td>
<td>normal to 1/4 X; round to oval; some fimbriated; Schuffner’s dots</td>
<td>compact with large chromatin; dark-brown pigment</td>
</tr>
<tr>
<td></td>
<td>Schizonte</td>
<td>normal to 1/4 X; round to oval; some fimbriated; Schuffner’s dots</td>
<td>mature = 6-14 merozoites with large nuclei, clustered around mass of dark-brown pigment</td>
</tr>
<tr>
<td></td>
<td>Gametocyte</td>
<td>normal to 1/4 X; round to oval; some fimbriated; Schuffner’s dots</td>
<td>round to oval; compact; may almost fill RBC; chromatin compact, eccentric (macrogametocyte) or more diffuse (microgametocyte); scattered brown pigment</td>
</tr>
<tr>
<td><strong>P. malariae</strong></td>
<td>Ring</td>
<td>normal to 3/4 X</td>
<td>sturdy cytoplasm; large chromatin</td>
</tr>
<tr>
<td></td>
<td>Trophozoite</td>
<td>normal to 3/4 X; rarely, Ziemann’s stippling (under certain staining conditions)</td>
<td>compact cytoplasm; large chromatin; occasional band forms; coarse, dark-brown pigment</td>
</tr>
<tr>
<td></td>
<td>Schizonte</td>
<td>normal to 3/4 X; rarely, Ziemann’s stippling (under certain staining conditions)</td>
<td>mature = 6-12 merozoites with large nuclei, clustered around mass of coarse, dark-brown pigment; occasional rosettes</td>
</tr>
<tr>
<td></td>
<td>Gametocyte</td>
<td>normal to 3/4 X; rarely, Ziemann’s stippling (under certain staining conditions)</td>
<td>round to oval; compact; may almost fill RBC; chromatin compact, eccentric (macrogametocyte) or more diffuse (microgametocyte); scattered brown pigment</td>
</tr>
</tbody>
</table>
### Keypoints for *Plasmodium* Species Which Cause Human Malaria

#### Infected RBCs

<table>
<thead>
<tr>
<th>Size</th>
<th>Shape</th>
<th>Schüffner’s Dots</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;N, N: PM</td>
<td>Crescent: PF (gametocyte)</td>
<td>PV, PO</td>
</tr>
<tr>
<td>N: PF</td>
<td>Ameboid: PV</td>
<td></td>
</tr>
<tr>
<td>&gt;N: PO</td>
<td>Fimbriated: PO</td>
<td></td>
</tr>
<tr>
<td>&gt; &gt;N: PV</td>
<td>Elongated: PO</td>
<td></td>
</tr>
</tbody>
</table>

#### Parasites Found In Circulating Blood

<table>
<thead>
<tr>
<th>Rings</th>
<th>Trophozoites</th>
<th>Schizonts (mature)</th>
<th>Gametocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rings only (s:gametocytes): PF</td>
<td>Ameboid: PV</td>
<td>6-12 nuclei: PM</td>
<td>Crescent: PF</td>
</tr>
<tr>
<td>Numerous: PF</td>
<td>Compact: PO</td>
<td>6-14 nuclei: PO</td>
<td>Round: PV</td>
</tr>
<tr>
<td>Multiply infected RBCs: PF</td>
<td>PM</td>
<td>12:24: PV</td>
<td>PO</td>
</tr>
<tr>
<td>Accessory chromatin dots: PF</td>
<td>PF (rarely seen)</td>
<td>8-24: PF (rarely seen)</td>
<td>PM</td>
</tr>
<tr>
<td>Delicate: PF</td>
<td>Band form: PM</td>
<td>Rosettes: PM</td>
<td></td>
</tr>
</tbody>
</table>

Certain morphologic key characteristics of the infected erythrocytes and parasites can be used to orient the diagnosis towards one of the four *Plasmodium* species that infect humans, as shown above. These characteristics are by no means absolute, however. The final diagnosis should be based on the combined findings for the various characteristics: what is the most probable species, based on the available findings.

### Legend

- PF: *P. falciparum*
- PV: *P. vivax*
- PO: *P. ovale*
- PM: *P. malariae*

---

Note: *P. Plasmodium*; RBC, red blood cell.
Annex 4: Preparation of blood films

**Laboratory diagnosis of malaria**

**Preparation of blood smears**

### Blood collection for thick or thin blood smears

**Capillary blood obtained by fingerstick:**

1. Label pre-cleaned slides (preferably frosted-end) with patient's name (or other identifier), date and time of collection.

2. Wear gloves.

3. Clean slides with 70 to 90% alcohol and allow to dry. Do not touch the surface of the slide where the blood smear will be made.

4. Select the finger to puncture, usually the middle or ring finger. In infants, puncture the heel.

5. Clean the area to be punctured with 70% alcohol; allow to dry.

6. Puncture the ball of the finger, or in infants puncture the heel.

7. Wipe away the first drop of blood with clean gauze.

8. Touch the next drop of blood with a clean slide. Repeat with several slides (at least two thick and two thin smears should be made). If blood does not well up, gently squeeze the finger.

**For venous blood obtained by venipuncture:**

1. Label collection tubes and pre-cleaned slides (preferably frosted-end) with the patient’s name (or other identifier), date and time of collection.

2. Clean the site for blood collection well using 70% alcohol; allow to dry.

3. Collect the venous blood in a vacuum tube containing anticoagulant (preferably EDTA); alternatively, collect the blood in a syringe and transfer it to a tube with anticoagulant; mix well.

4. Prepare at least two thick smears and two thin smears as soon as possible after collection.
Annex 5: Staining for malaria parasites

Laboratory diagnosis of malaria

Making thick and thin blood smears

1. Whenever possible, use separate slides for thick and thin smears.

2. Thin film (a): Bring a clean spreader slide, held at a 45° angle, toward the drop of blood on the specimen slide.

3. Thin film (b): Wait until the blood spreads along the entire width of the spreader slide.

4. Thin film (c): While holding the spreader slide at the same angle, push it forward rapidly and smoothly.

5. Thick film: Using the corner of a clean slide, spread the drop of blood in a circle the size of a dime (diameter 1-2 cm). Do not make the smear too thick or it will fall off the slide. (You should be able to read newsprint through it.)

6. Wait until the thin and thick films are completely dry before staining. Fix the thin film with methanol (100% or absolute) and let it dry completely before staining. The thick film should not be fixed.

7. If both thin and thick films need to be made on the same slide, fix only the thin film with methanol. The thick film should not be fixed.

For a video of how to make a thick smear, please visit the DPDx website at:
http://www.dpd.cdc.gov/dpdx/HTML/Frames/DiagnosticProcedures/body_dp_bloodthickavi.htm

For a video of how to make a thin smear, please visit the DPDx website at:
Staining Blood Smears
Stain only one set of smears, and leave the duplicates unstained. The latter will prove useful if a problem occurs during the staining process and/or if you wish later to send the smears to a reference laboratory.

Giemsa stain - Recommended for detection and identification of blood parasites.

1. Stock 100x Giemsa Buffer 0.67 M
   Na₂HPO₄ 59.24 g
   NaH₂PO₄·H₂O 36.38 g
   Deionized water 1000.00 ml
   Autoclave or filter-sterilize (0.2 µm pore). Sterile buffer is stable at room temperature for one year.

2. Working Giemsa Buffer 0.0067M, pH 7.2
   Stock Giemsa Buffer 10.0 ml
   Deionized water 990.0 ml
   Check pH before use. Should be 7.2. Stable at room temperature for one month.

3. Triton X-100 5%
   Deionized water (warmed to 56°C) 95.0 ml
   Triton X-100 5.0 ml
   Prewarm the deionized water and slowly add the Triton X-100, swirling to mix.

4. Stock Giemsa stain (Giemsa stain is available commercially, but the following formulation gives more constant results and does not expire.)
   Glass beads, 3.0 mm 30.0 ml
   Absolute methanol, acetone-free 270.0 ml
   Giemsa stain powder (certified) 3.0 g
   Glycerol 140.0 ml
   • Put into a 500 ml brown bottle the glass beads and the other ingredients, in the order listed. Screw cap tightly. Use glassware that is clean and dry.
   • Place the bottles at an angle on a shaker; shake moderately for 30 to 60 minutes daily, for at least 14 days.
   • Kept tightly stoppered and free of moisture, stock Giemsa stain is stable at room temperature indefinitely (stock stain improves with age).
   • Just before use, shake the bottle. Filter a small amount of this stock stain through Whatman #1 filter paper for use as the working Giemsa stain.

5. Working Giemsa stain (2.5%): make fresh daily. If a large number of smears are made, stain may need to be changed throughout the day.
   Working Giemsa buffer 40 ml
   Giemsa Stain Stock 1 ml
   5% Triton X-100 20 µl (equivalent to 2 drops)
Annex 6: Determination of parasitemia

Determination of Parasitemia

Determination of parasitemia can be done using both thick and thin smears.

**Thick smears:**
The number of parasites/μl of blood is determined by enumerating the number of parasites in relation to the standard number of WBCs/μl (8000).

\[
\text{No. Parasites} \times (8000 - \text{No. WBCs counted}) \\
= \text{No. parasites per } \mu\text{L of blood}
\]

**Thin smears:**
The percent of infected RBCs is determined by enumerating the number of infected RBCs in relation to the number of uninfected RBCs. A minimum of 500 RBCs total should be counted.

\[
(\text{No. infected RBCs} - \text{Total No. RBCs counted}) \times 100 \\
= \text{Percent Infected RBCs}
\]

Notes:
- Multiply infected RBCs are counted as one.
- Gametocytes are not figured in calculations.

Note: No., number; RBC, red blood cell; WBC, white blood cell.
How To Do the Rapid Test for Malaria
Modified for training in the use of the Generic PI Test for falciparum malaria

Collect:
- NEW unopened test packet
- NEW unopened alcohol swab
- NEW unopened lancet
- NEW pair of disposable gloves
- Buffer
- Timer

READ THESE INSTRUCTIONS CAREFULLY BEFORE YOU BEGIN.

1. Check the expiry date on the test packet.
2. Put on the gloves, the new gloves for each patient.
3. Open the packet and remove:
   a. Test
   b. Capillary tube
   c. Alcohol swab
4. Write the patient’s name on the test.

5. Open the alcohol swab. Grasp the 4 fingers on the patient’s left hand. Clean the finger with the alcohol swab. Allow the finger to dry before pricking.
6. Open the lancet. Prick patient’s finger to get a drop of blood.
7. Discard the lancet in the sharps box immediately after pricking fingers. Do not set the lancet down before discarding it.
8. Use the capillary tube to collect the drop of blood.
9. Use the capillary tube to put the drop of blood into the square hole marked “A.”
10. Discard the capillary tube in the sharps box.
11. Add buffer into the round hole marked “B.”
12. Wait 15 minutes after adding buffer.
13. Read test results.
   - **POSITIVE**: A line near letter “C” and a line near letter “T” means the patient is POSITIVE for malaria.
   - **NEGATIVE**: A line near letter “C” and NO LINE near letter “T” means the patient DOES NOT have malaria.
   - **INVALID RESULT**: No line near letter “C” and one or no line near letter “T” means the test is INVALID.

14. How to read the test results.

15. Dispose of the gloves, alcohol swab, desiccant packet and packaging in a non-sharp waste container.
16. Record the test results in your CMS register. Dispose of waste in non-sharp waste container.

NOTE: Each test can be used ONLY ONE TIME. Do not try to use the test more than once.
Annex 8: Template for thick and thin blood films\textsuperscript{1}

Thick film template (circle) is 1.2 cm in diameter and takes 6 μL of blood, spread evenly to edge of circle.
