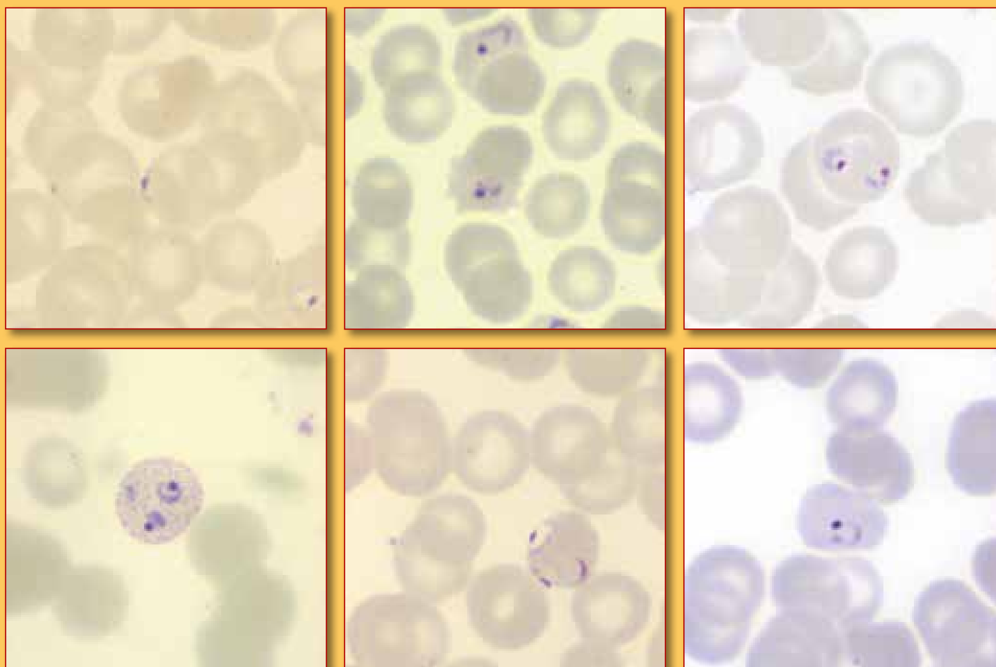
MALARIA MICROSCOPY

Quality Assurance Manual



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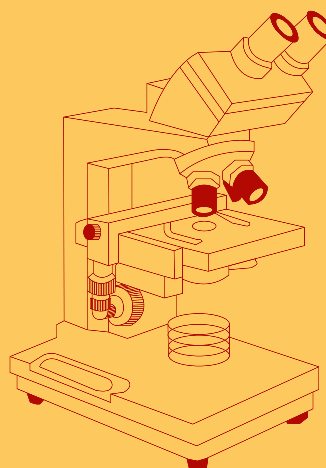

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Front cover, inserts : photomicrographs of Giemsa stained thin films showing clockwise from top left : early trophozoites (ring stages) of 1) *Plasmodium falciparum*, 2) *Plasmodium vivax*, 3) *Plasmodium malariae* and 4) *Plasmodium ovale*; and mature trophozoites of 5) *Plasmodium falciparum* and *Plasmodium vivax*.

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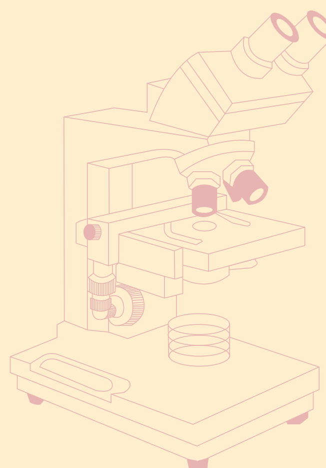

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Abbreviations

ACTMalaria	Asian Collaborative Training Network for Malaria
ECA	external competence assessment
EDTA	ethylenediaminetetraacetic acid
JSB	Jaswant Singh Battacharya
NCA	national competence assessment
NGO	nongovernmental organization
NMCP	national malaria control programme
NRL	national reference laboratory
OTSS	outreach training and supportive supervision
PCR	polymerase chain reaction
QA	quality assurance
QC	quality control
RBC	red blood cell
RDT	rapid diagnostic test
SOP	standard operating procedure
WBC	white blood cell

Preface

The first version of the WHO *Malaria microscopy quality assurance manual* (2009) was based on recommendations made at a series of informal consultations organized by WHO, particularly a bi-regional meeting of the WHO regional offices for South-East Asia and the Western Pacific in April 2005 in Kuala Lumpur, Malaysia, followed by informal consultations held in March 2006 and February 2008 in Geneva, Switzerland. Subsequently, extensive consultations among international malaria experts led to consensus and preparation of the manual. This second version of the *Manual* is based on the recommendations of experts made at a WHO technical consultation in March 2014 in Geneva, Switzerland. The aim of the meeting was to review the experiences of national malaria control programmes (NMCPs), national reference laboratories (NRLs) and technical agencies in using the *Manual* and country experience in order to improve systems for managing the quality of malaria microscopy.

This second version takes into account the many years of experience of several agencies in the various aspects of quality assurance (QA) described in the *Manual*. In particular, the sections on assessment of competence in malaria microscopy are based on use of this method by the WHO regional offices for South-East Asia and the Western Pacific, in collaboration with the WHO Coordinating Centre for Malaria in Australia, and by the WHO Regional Office for Africa in collaboration with Amref Health Africa. The section on setting up and managing an international reference malaria slide bank is based on the work of the WHO Regional Office for the Western Pacific in collaboration with the WHO Coordinating Centre for Malaria Diagnosis in the Philippines. The section on proficiency testing for malaria microscopy is based on work in the WHO Regional Office for Africa in collaboration with the National Institute for Communicable Diseases in South Africa and experience in regional initiatives by Amref Health Africa. The section on slide validation is based on work by Médecins sans Frontières, and the section on outreach training and supportive supervision (OTSS) is based on work by the President's Malaria Initiative Malaria Care Project, Medical Care Development International and Amref Health Africa.

Before finalization the manual was field tested at the EMRO Regional Training Course on Quality Assurance of Malaria Diagnosis, held at the Blue Nile National Institute for Communicable Diseases, Wad Madani, Gezira Stat, Sudan, from 24 October to 6 November 2015.

The *Manual* is designed primarily to assist managers of NMCPs and general laboratory services responsible for malaria control. The information is also applicable to nongovernmental organizations (NGOs) and funding agencies involved in improving quality management systems for malaria microscopy.

The *Manual* is not designed for QA of microscopy in research situations, such as in clinical trials of new drugs and vaccines, or for monitoring parasite drug resistance. It forms part of a series of WHO documents designed to assist countries in improving the quality of malaria diagnosis in clinical settings, including the revised training manuals on *Basic malaria microscopy* (2010) and the *Bench aids for malaria microscopy* (2010).

Note on use of the term "microscopist"

Malaria programmes in different countries and regions use various terms to denote a person who uses a microscope to read blood films in order to diagnose malaria and report their findings. This may be done in many contexts, including case management in small rural clinics, as part of a teaching curriculum in a university or to provide a reference standard in a large clinical trial. It may be just one of the duties of a senior laboratory consultant, a scientist or technician in a reference laboratory or the entire workload of a staff member in a small outpatient clinic. In this *Manual*, the term is used to denote any person who carries out such an activity, as the principles discussed apply to various degrees to personnel who perform this task at multiple levels of the health care system.

Definition of "quality assurance"

QA of a malaria laboratory or diagnostic programme is designed to improve the efficiency, cost–effectiveness and accuracy of test results continuously and systematically. The primary objectives of QA are to ensure that:

- health care professionals and patients have full confidence in the laboratory result and
- the diagnostic results benefit the patient and the community.

These objectives can be achieved only by a commitment to QA to ensure that microscopy services are staffed by competent, motivated staff, supported by effective training and supervision. A logistics system is required to ensure an adequate, continuous supply of good-quality reagents and essential equipment maintained in working order. The facilities should be subjected regularly to external quality assessment.

The principles and concepts of QA for microscope diagnosis of malaria are similar to those for microscope diagnosis of other communicable diseases, such as other protozoan diseases, tuberculosis and helminth infections. Therefore, QA for laboratory services should be integrated wherever it is feasible and cost–effective.

Executive summary

Early diagnosis and prompt effective treatment are the basis for the management of malaria and for reducing malaria mortality and morbidity. Demonstration of the presence of malaria parasites before treatment with antimalarial drugs is fundamental to this goal, as the accuracy of clinical diagnosis is poor, leading to over-diagnosis of malaria, poor management of non-malarial febrile illness and wastage of and increasing resistance to antimalarial drugs. While microscopy remains the mainstay of parasite-based diagnosis in most large health clinics and hospitals, the quality of microscopy-based diagnosis is frequently inadequate to ensure good health outcomes and optimal use of resources.

An acceptable microscopy service is one that is cost–effective and provides results that are consistently accurate and timely enough to have a direct impact on treatment. This requires a comprehensive, active QA programme.

The aim of malaria microscopy QA programmes is to ensure that microscopy services provide accurate results; are administered by competent, motivated staff supported by effective training, supervision and quality control (QC) to maintain their competence and performance; and are supported by a logistics system to provide and maintain adequate supplies of reagents and equipment. QA programmes must be:

- sustainable,
- compatible with the needs of the country and
- able to fit into the structure of existing laboratory services.

A QA programme should appropriately recognize good performance; identify laboratories and microscopists with serious problems that result in poor performance; establish regional or national benchmarks for the quality of diagnosis; and ensure central reporting on indicators, including accuracy, equipment and reagent performance, stock control and workload.

This *Manual* is designed primarily for use by managers of NMCPs and health facilities with laboratory services, to support them in setting up and maintaining a sustainable malaria microscopy QA programme. It outlines a hierarchical structure based on re-training, cross-checking and standards of competence, which is designed to ensure the quality of diagnosis necessary for a successful malaria programme, with reasonable levels of financial and human resources. Without an efficient QA programme, resources spent on diagnostic services are likely to be wasted and clinicians will lose confidence in the results provided by malaria microscopy.

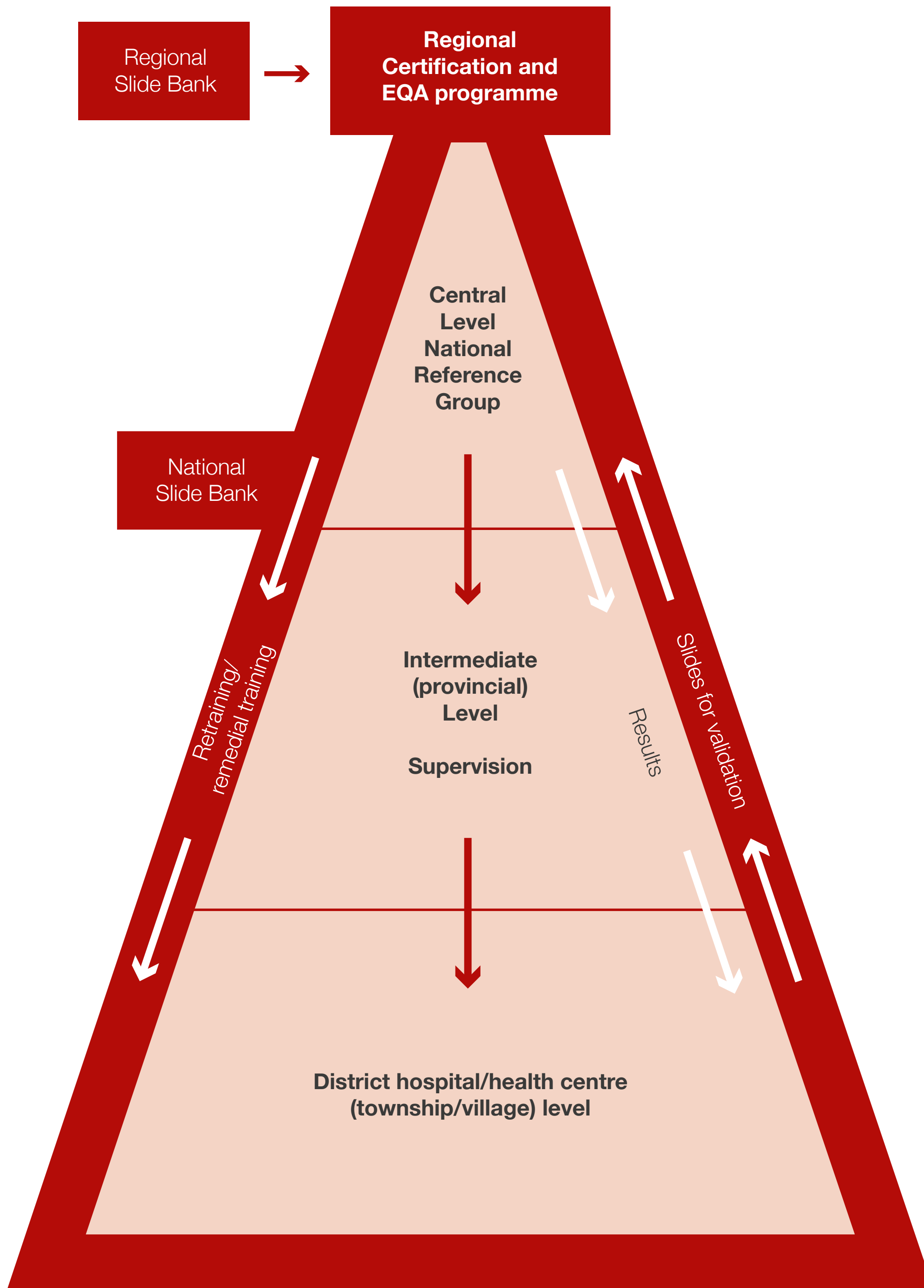
The QA system outlined in this *Manual* should be adapted to the national context of laboratory services that provide malaria microscopy. These may be under the NMCP or a separate institution working closely with the malaria programme. The microscopists may be formally trained laboratory scientists, technicians working in tertiary health services conducting a range of specialized diagnostic activities or health workers trained in malaria microscopy with or without other laboratory roles. In all cases, the principles remain the same.

At a minimum, a malaria microscopy QA programme should have:

- a central coordinator(s) to oversee QA. This position is essential, as the QA programme requires constant coordination and advocacy to be effective;
- a reference (core) group of microscopists at the head of a hierarchical structure, supported by an external QA programme, with demonstrable expertise in overseeing programme training and validation standards;
- good initial (pre-service) training with competence standards that must be met by trainees before they work in a clinical setting;
- clear SOPs at all levels of the system;
- regular refresher (in-service) training and assessment of competence, supported by a well-validated reference slide set (slide bank);
- a sustainable cross-checking system to detect gross inadequacies without overwhelming "validators" higher up in the structure, with good, timely feedback of results and a system to correct inadequate performance;
- regular, effective, structured supervision at all levels;
- efficient, effective logistical management, including supplies of consumables and maintenance of microscopes and other equipment; and
- an adequate budget for funding the above activities.

This *Manual* describes the essential elements necessary to establish this structure.

Figure 1. Structure and function of the quality assurance system



Glossary

Administrative level (of laboratory services)

Laboratory services are usually organized into three main levels: the national or central level, a regional, provincial or intermediate level, and a district health centre or peripheral level. Laboratory services at the national level might be an integral part of the NMCP, part of the general health services or a suitably designated NRL. Peripheral laboratory services are often primary diagnostic facilities in peripheral health facilities for outpatients; in some settings, they may include microscopy services at village level, operating within health posts.

Artemisinin-based combination therapy

A combination of an artemisinin derivative with a longer-acting antimalarial agent that has a different mode of action.

Benchmarking

A comparison of the performance of all laboratories and/or test centres in a programme on the basis of standardized indicators, e.g. comparison of the performance of laboratories in a QC programme.

External quality assessment

A system by which a laboratory's performance is checked objectively by an external agency or facility or a reference laboratory.

False negative

A positive blood smear that is misread as negative.

False positive

A negative blood smear that is misread as positive.

Feedback

Communication of the results of proficiency testing or external quality assessment to the original laboratory, with identification of errors and recommendations for remedial action.

First- and second-line antimalarial drugs

First-line antimalarial medicines are those recommended in national treatment guidelines for treating uncomplicated malaria. Second-line drugs are those used to treat treatment failures after use of first-line drugs.

Microscopist

A person who uses a microscope to read blood films to assist or confirm a diagnosis of malaria and who reports the findings. The term is used in this *Manual* to include personnel at all levels of a malaria programme involved in such work, from professors involved in teaching and research to village health volunteers specifically trained in malaria microscopy.

National malaria control programme

The countrywide programme responsible for all activities related to the prevention, control and elimination of malaria. These include activities integrated with general health services to provide diagnosis and treatment for malaria.

National reference or central laboratory

This may be part of the central public health laboratory, the NMCP or a government institution in academia. It plays an essential role in the preparation of guidelines for standardizing methods, maintaining slide banks, producing locally adapted training materials, providing basic and refresher training, overseeing training activities, assuring the quality of testing and supporting external QA in collaboration with the NMCP.

Performance standard

A level of performance that is considered acceptable and that all laboratories and test centres should meet or exceed. Performance standards make it possible to identify laboratories that are not performing satisfactorily.

Proficiency testing

A system in which a reference laboratory sends blood films to a laboratory for examination, and the laboratory receiving the slides is not informed of the correct results until it has reported its findings back to the reference laboratory.

Quality assurance

The maintenance and monitoring of the accuracy, reliability and efficiency of laboratory services. QA addresses all the factors that affect laboratory performance, including test performance (internal and external QC), the quality of equipment and reagents, workload, workplace conditions, training and supervision of laboratory staff and continuous quality improvement. It includes procedures put in place to ensure accurate testing and reporting of results.

Quality control

Assessment of the quality of a test or a reagent. QC also encompasses external QC and reagent QC. **External QC** is a system in which routine blood slides are cross-checked for accuracy by a supervisor or the regional or national laboratory. **Reagent QC** is a system for formal monitoring of the quality of the reagents used in a laboratory.

Quality improvement

A process in which the components of microscopy and RDT diagnostic services are analysed in order to identify and permanently correct any deficiencies. Data collection, data analysis and creative problem-solving are used.

Rapid diagnostic test

Rapid diagnostic tests (RDTs) are immuno-chromatographic tests for detecting parasite-specific antigens in blood samples. Some malaria RDTs detect only one species *(P. falciparum* or *P. vivax)*, while others detect *P. falciparum* with one or more of the other three species of human malaria parasite (*P. vivax, P. malariae* and *P. ovale*). RDTs are commercially available in different formats, as dipsticks, cassettes or cards.

Slide positivity rate

The proportion of positive results, detected by microscopy, among all slides examined over a defined period.

SMART indicators

Indicators of performance that are Specific, Measurable, Achievable, Attainable, Realistic and Timely.

1. WHY QUALITY ASSURANCE OF MALARIA MICROSCOPY SHOULD BE IMPROVED

The detection of malaria parasites by light microscopy remains the reference method for diagnosis of malaria throughout the world. This requires a reliable microscopy service that:

- is cost effective,
- is accurate and timely and
- gives results with a direct impact on the treatment given to a patient.

The effectiveness of malaria microscopy depends on maintaining a high level of staff competence and performance, ensuring good-quality reagents and equipment at all levels and regular external assessment.

1.1 Accurate diagnosis

The first suspicion of malaria is usually based on clinical criteria, especially fever or a recent history of fever; however, even in areas of high transmission, most cases of fever are usually not due to malaria. As the clinical manifestations of malaria are non-specific, a diagnosis based on clinical symptoms alone results in a high number of false-positive results; often, other diseases are overlooked or not treated in a timely manner, contributing to significant morbidity and mortality due to non-malaria illness. False-positive results also lead to misuse of antimalarial drugs, exposure of parasites to sub-therapeutic blood levels of the drugs and development of resistance, increased costs to the health services and patient dissatisfaction.

An accurate laboratory diagnosis is essential, as false-negative results can lead to untreated malaria and potentially severe consequences, including death. False-negative results can also significantly undermine both clinical confidence in laboratory results and the credibility of the health services within a community.

Parasitological confirmation of malaria is critical not only for case management but also for accurate measurement of the malaria burden.

Since 2010, WHO has recommended that all suspected cases of malaria be confirmed parasitologically by microscopy or RDTs before treatment, irrespective of age and transmission setting. The exception to this rule is when confirmatory tests are unavailable or are known to be of poor quality.

1.2 Role of light microscopy in current malaria control and elimination strategies

Microscope diagnosis has many advantages, including:

- low direct costs if there is already a high volume of samples and the infrastructure to maintain the service;
- highly sensitive for clinical malaria, if the quality of microscopy is good (including competent microscopists, good equipment and reagents and an appropriate workload), although not sensitive for detecting low-density parasitaemia;
- allows differentiation of malaria species and parasite stages;
- allows determination of parasite density;
- allows assessment of drug effects; and
- can be used to diagnose other diseases.

Blood film microscopy remains the only inexpensive, easily used test for direct measurement of the presence of parasites, distinguishing the infecting parasite species and providing a means of quantifying parasite load. These characteristics of malaria microscopy make it an invaluable tool in the control of malaria, including for studies of therapeutic efficacy, which depend on good-quality microscopy.

If microscopy services cannot be extended to confirm all cases of suspected malaria, it should be used to detect the presence of parasites in all cases of suspected treatment failure and severe disease.

1.3 Promotion of microscopic diagnosis of malaria

Accurate microscopy results depend on the availability of a competent microscopist using good-quality reagents for examining well-prepared slides under a well-maintained microscope with an adequate light source and with a low-to-moderate workload. It has therefore been difficult to maintain good-quality microscopy, especially in peripheral health services, where most patients seek treatment. The private sector, which also provides laboratory services to a large part of the population in some countries, often remains severely under-regulated.

The factors that limit the availability and quality of microscopy include:

- lack of resources to provide all laboratories with equipment and good-quality reagents for microscopy;
- absence of effective pre-service training;
- lack of programmes and resources for training and continuous improvement of the competence of microscopists;
- lack of SOPs;
- difficulty in maintaining microscopy facilities in good order and lack of microscope maintenance capability;
- lack of electricity, water and suitable laboratory facilities;
- logistical problems and high costs of maintaining adequate supplies and equipment;
- lack of a QC system at central level for supplies, reagents and equipment before distribution;
- lack of national malaria slide banks for building and monitoring competence;
- absence of a national system to certify the level of competence of microscopists and career pathways;
- heavy workloads, which delay the provision of results to clinical staff;
- weak supervision of laboratory services and lack of remedial action;

- inability to cope with the workload of cross-checking routine malaria slides, often due to inadequate human and financial resources;
- limited participation in external QA systems and application of remedial actions;
- lack of an internal QC system, particularly in peripheral laboratories; and
- decreasing practice of malaria microscopy in some settings because of extensive deployment of RDTs and fewer positive cases after a reduction in the malaria burden.

These limitations can be overcome only by new health policies based on acknowledgement of the importance of strengthening laboratory services and mobilization of adequate funding for implementation of a QA system to ensure:

- continuous training, assessment and supervision of microscopists and QC of their tasks;
- regular supportive supervision and mentoring at health facilities;
- accurate, timely blood collection, slide staining and reading linked to clinical diagnosis;
- rapid provision of results to clinicians;
- clinicians trusting the results;
- logistical support to ensure good-quality supplies and equipment; and
- the sustainability of the QA programme, with adequate staff and resources.

As malaria is a disease that disproportionally affects the poorest countries, programmes must decide realistically where high-quality microscopy can be maintained and where it is more feasible to rely on RDTs for diagnosis of fever.

1.4 Improving the competence and performance of microscopists

In many countries endemic for malaria, microscopists receive initial training and are assumed to be competent for the rest of their careers. There are very few structured refresher courses or other means of enhancing and updating skills. Refresher courses and more advanced training are means of continuous education and are often provided ad hoc without consideration of need. Laboratory managers often attend refresher training, although they generally do not routinely diagnose malaria.

In some settings, malaria microscopists do not even receive formal training and are expected to learn on the job from others, who often do not have the requisite skills and tools to train. Thus, microscopists with little competence often teach others, who in turn acquire less skill, feeding a cycle of low quality.

High competence and performance are achieved when microscopists at all levels are supported by continuous training and assessment, with refresher training when required, according to international standards. Although such standards apply primarily to national programme staff and trainers, they should also be applicable to staff working with NGOs and in the private sector. Countries should set standards to ensure that all participants enrolled in a training course have the appropriate experience and responsibility in clinical microscopy and will be able to apply their new skills.

When QA programmes for malaria microscopy are not adequate, priority should be given to training and assessing senior microscopists at central and intermediate levels, as it is them who will be responsible for the training and assessment of peripheral staff.

1.4.1 Defining competence and performance

Competence in microscopy is the ability of a microscopist to examine a malaria blood film accurately and report the results accurately. Competence also includes the ability of a microscopist to identify and correct problems in preparing, fixing or staining blood films.

Measuring competence requires:

- definition of the specific educational requirements and skills required at each level of the QA system;
- setting standards of competence;
- standardized training materials and courses;
- regular scheduled assessments; and
- standardized, objective assessment at the end of training.

Competence can be improved by:

- refresher training,
- supervision and
- regular exposure to blood film microscopy.

Performance in microscopy is a measure of the correctness of output (accuracy of diagnosis and reporting) of the microscopist in routine practice.

Measuring the performance of a microscopist requires:

- clear definition of performance standards;
- standardized, unbiased cross-checking of a sample of slides routinely examined by the microscopist;
- participation in a proficiency testing scheme; and
- monitoring of performance.

Performance can be improved by:

- providing SOPs, job aids and QA manuals;
- providing and maintaining good-quality microscopes, stains and supplies;
- ensuring a reasonable, managed workload;
- support and mentoring visits by supervisors;
- effective responses to problems by both supervisors and microscopists, including targeted retraining or equipment maintenance;
- periodic refresher training; and
- motivation by positive reinforcement from supervisors, personal certification of all supervisors and microscopists and opportunities for career advancement.

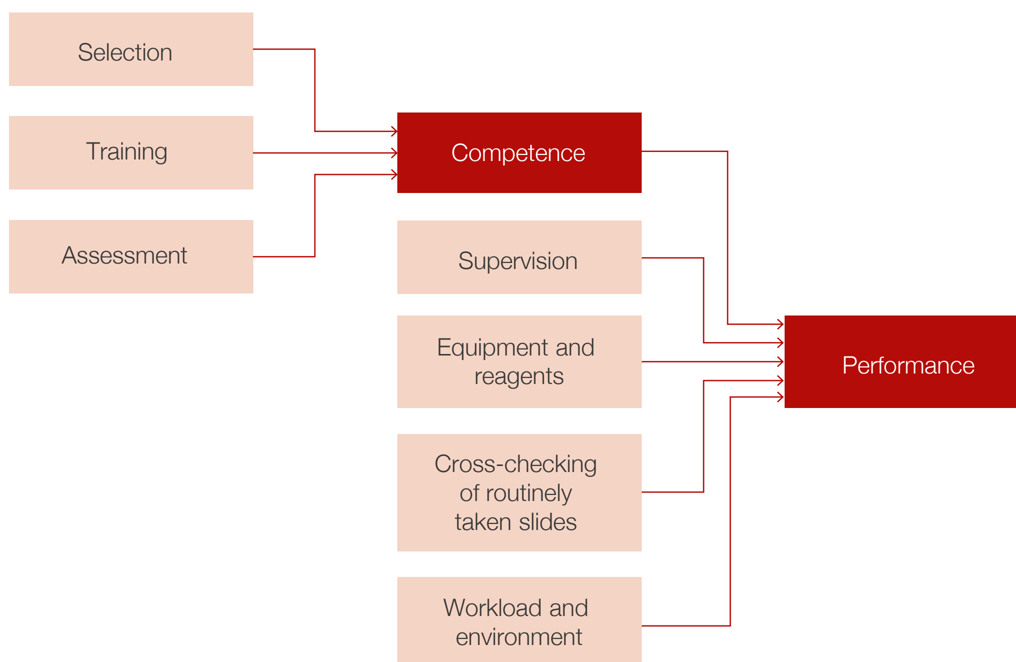
1.4.2 Assessing the performance of malaria microscopy

The performance of malaria microscopy must be monitored continuously in a QA programme, based on predefined standards. QA has two essential components:

- assessment of the quality of blood-film preparation and the accuracy of thick and thin blood film examinations for malaria diagnosis and for monitoring the response to treatment, either during visits from supervisors or by external blinded cross-checking of slides; and
- monitoring systems to assess staff competence, facilities and equipment, reagents, stock control, workload, registration and reporting.

The primary aim of basic QA programmes is to identify laboratories practices and individuals that have deficiencies that adversely affect the final result of a test. The ultimate goal is to introduce practices that consistently lead to good-quality results and ensure that laboratories can identify and resolve problems in malaria diagnostics. QA should be incorporated into medium-term planning for programmes starting from a low baseline; programmes with a more developed infrastructure should use the most comprehensive QA system possible. National or regional programmes should prepare minimum acceptable standards and quality indicators. The relations between competence and performance are illustrated in Fig. 2.

Figure 2. Ensuring and demonstrating good performance in malaria microscopy



A comprehensive malaria QA programme will include all of the following:

- baseline assessments to identify gaps in the QA system,
- training (initial and refresher),
- on-site supervision with corrective training and problem-solving,
- slide rechecking,
- competence assessment,
- proficiency testing,
- equipment and reagent quality control and maintenance and
- effective remediation of deficiencies.

2. STRUCTURE AND FUNCTION OF A QUALITY ASSURANCE SYSTEM

2.1 Why quality assurance systems should be expanded

The QA systems for diagnosis of malaria by microscopy comprise all the processes necessary to ensure that the result is as accurate as microscopy allows, from blood collection to delivery of the results. Strengthening QA has become a priority with the reduction in the prevalence of malaria as a result of effective interventions and in order to distinguish malaria from non-malarial fevers.

Some QA programmes are incomplete or ineffective because of neglect and lack of funding. They cannot be upgraded without additional financial investment and human resources. Some countries might be able to mobilize national resources, but many others will require assistance from the international community. Regardless of the sources of investment, national programmes must prepare realistic proposals with credible budgets indicating value for money to convince decision-makers that they could benefit from investing in building the infrastructure and human resources required to ensure good-quality malaria microscopy. If a programme has to be rebuilt, it will have to be according to a phased plan of action that covers at least 5 years as part of the country's national strategic plan for malaria.

2.2 Basic structure

WHO has recommended for many years that malaria microscopy and QA be integrated with other programmes for communicable diseases that are diagnosed microscopically, when they are compatible. Thus, in countries where malaria microscopy is performed in the general health services, the malaria QA programme should be the responsibility of the national laboratory services with technical support from the NMCP, in collaboration with other institutions in the country that conduct QA, such as universities, the NRL and NGOs. Such a combined system will:

- simplify the administration, logistics of supply of reagents and equipment, reporting and evaluation of the performance of microscopy;
- require fewer resources, as QA for malaria could use the resources and infrastructure of other QA schemes;
- contribute to improving other laboratory services, including use of new, validated tests, by strengthening the supply chain for reagents and equipment and the maintenance of microscopes and other equipment;
- allow optimal use of microscopes and other equipment in laboratories with low workloads;
- promote a common proficiency system in laboratories with low workloads;
- develop interesting initiatives for microscopists to increase their motivation;
- provide a harmonized competence assessment scheme that could be linked to career development;
- require a single budget;
- simplify monitoring and evaluation, resulting in a more transparent system; and
- leverage resources from multiple donors.

In countries in which there is no national laboratory service or one that does not function adequately, the ministry of health, through the NMCP, should take the responsibility for setting up a malaria microscopy QA system, in collaboration with the general health services and other interested partners, with the long-term goal of integrating malaria QA into general health services, as conditions allow.

A malaria microscopy QA programme should be implemented in a phased approach, with emphasis on sustainable, regular on-site supervision and periodic refresher training. The starting-point should be the central level, with a national reference group. Section 2.2.1 lists the functions to be coordinated at that level. One of the first tasks will be to improve the competence of microscopists, with standardized assessment, as they will be involved in important aspects of QA, including formal and outreach training, cross-checking malaria slides, supervisory visits, coordinating the proficiency testing programme, preparing SOPs, setting up reference slide banks and preparing bench aids. As the QA programme develops, it will move to the intermediate and peripheral levels. The relation of this structure to functions at the different levels is shown in Fig. 1, page XIII.

The common hierarchical organization of general laboratory services into national (central), provincial, state or regional (intermediate) and district or health centre (peripheral) laboratories is ideal for the management and operation of a QA system. The increasing complexity of performance standards and responsibilities from the peripheral to the central level could facilitate career advancement for microscopists. This is important, as it will make microscopy more attractive for people entering the service and provide an incentive for those already in service

2.2.1 Central level

The central level ensures the quality of diagnosis at all levels; it is usually responsible for planning, implementing and monitoring QA nationwide. The level could be represented by a laboratory within the general laboratory services of the ministry or department of health, associated with a large hospital or a research institute, or a national laboratory within the NMCP. Irrespective of the arrangement, a competent laboratory must be designated as the NRL, with which the NMCP will collaborate and coordinate.

The NRL should participate in an international certification programme (such as the WHO Malaria Microscopy External Competence Assessment) that includes recognition and certification of the expertise of its staff. Retraining and certification are essential to ensure expertise and to contribute to the expertise of the NRL for training and slide validation within the national QA system.

The NRL is responsible for establishing national standards for malaria diagnosis and for:

- pre-service and in-service training courses;
- preparing or adapting training materials for local situations and in local languages;
- assessing the competence and performance of microscopists according to WHO standards;
- national certification of microscopists;
- SOPs for laboratory testing and equipment; and
- SOPs for transport and storage of laboratory supplies and reagents.

The NRL could also be the focal point for international contacts and should strive for international and regional recognition as a centre of excellence. All staff at the NRL should have appropriate training and experience and demonstrable commitment to high standards of scientific practice and laboratory management.

2.2.2 Intermediate (provincial, state or regional) level

Microscopists at this level should be responsible for the supervision and QA of activities in order to maintain the quality of their laboratories. They should conduct external cross-checking of slides and:

- provide feedback on microscopy results and resolve identified problems;
- plan and conduct refresher training and supervision; and
- ensure that equipment is maintained in good working order, that there are no breakdowns in the supply chain, and that kits and reagents such as RDTs and Giemsa stain are stored and used according to the appropriate SOPs.

2.2.3 Peripheral (district, township or village) level

Depending on the country, laboratory services at this level may be organized at:

- primary diagnostic facilities in small, fixed health centres receiving mainly outpatients;
- mobile clinics or health posts attached to peripheral clinics;
- community level, with a village microscopist; or
- secondary diagnostic facilities, such as laboratories in hospitals and large health centres that receive both inpatients and outpatients

2.3 Quality assurance coordinator

Effective management by trained, competent senior staff is essential for the introduction and success of all QA programmes.

A national focal point should be appointed who has a clear mandate to oversee implementation of the QA programme. This national QA co-coordinator or manager should be a senior laboratory technologist, scientist or equivalent working at the central offices of the ministry or department of health or the NRL. He or she should be responsible for integrating malaria QA with other disease programmes when applicable.

The QA coordinator should be able to demonstrate that:

- quality-assured laboratory services have immediate benefits for improving case management of malaria;
- he or she can plan, implement and supervise programmes that are feasible, sustainable and compatible with the needs of the country; and
- she or he can prepare appropriate annual work plans and advocate for necessary funding.

This will require:

- a clear definition of the role and importance of the laboratory services in the planning and management of malaria control activities;
- recognition by the leadership of the ministry of health of the importance of laboratory diagnosis in malaria control;
- commitment to improve competence and performance at all levels of the laboratory services by regular refresher training, supervision and competence assessment of staff, including establishment of a national core group of certified, highly competent microscopists;
- ensuring feedback and continuous dialogue among all levels of the laboratory network;
- effective follow-up of poor performance, with appropriate remedial action, supportive supervision, problem-solving and continuing education;
- ensuring that all staff have a sense of ownership and responsibility;

- benchmarking to compare all the laboratories in the network and individual laboratories over time;
- a cost–effective plan of action with a realistic timetable and a budget commensurate with the activities to be carried out; and
- identification of a group of malaria diagnostic experts to advise and assist the NMCP and the ministry of health in making decisions and validating laboratory procedures.

2.4 Functional elements of the programme

The essential components of an effective malaria microscopy QA programme are similar for countries intending to control or to eliminate malaria; however, the aims of the programmes will be different. This *Manual* does not differentiate the QA requirements of control and elimination in countries, which are discussed in other documents. The essential functional elements of each QA programme are:

- a realistic plan of action prepared on the basis of a situation analysis;
- a budget commensurate with the plan of action, including adequate funding for all levels of the programme;
- a network of laboratories and microscopists to implement the programme, including a NRL or centre for preparing SOPs, bench aids and training and reference materials such as a slide bank;
- a programme for selection, training, retraining and assessment to ensure a competent workforce of laboratory staff, trainers and supervisors;
- a support network to ensure that the performance of the microscopists is maintained at the required level, including:
  - a QC system based on cross-checking and regular supervisory visits, particularly at the start of the programme and for laboratories found to be performing poorly;
  - an effective logistics system for the transport, storage and maintenance of essential supplies, reagents and equipment;
  - regular internal QC of routine laboratory operations;
  - a system to maintain equipment, particularly microscopes, in working order; and
- a monitoring system to ensure that standards are maintained and a culture of quality is present throughout the QA programme.

2.5 Tasks of microscopists

2.5.1 Malaria diagnosis

The job descriptions of staff at all levels of the QA programme should clearly state their responsibilities and define their tasks. The minimum areas of competence of a basic malaria microscopist are listed in Table 1.

Table 1. Minimum competence required of a basic malaria microscopist

Competence required
Blood film preparation
Cleaning of microscopy slides
Blood collection
Preparation of thick and thin films
Storage of stained slides
Staining
Correct dilution, quality testing and use of prepared stock of Giemsa stains
Correct preparation, quality testing and use of Field or Jaswant Singh Battacharya (JSB) stain[a] (if used)
Microscope
Basic cleaning and maintenance
Correct set-up (including correct illumination)
Correct use
Slide examination
Differentiate negative and positive slides
Accurately identify asexual stages
Accurately differentiate between P. falciparum and non-P. falciparum
Identify all species present in the region
Identify gametocytes
Count parasites
Identify all white blood cells (WBC)
Conduct a basic differential count on a thick film of neutrophils, monocytes, lymphocytes, eosinophils and basophils
Identify other major local blood parasites
Identify artefacts
Data
Record results in a laboratory register
Collate data regularly
Other
Basic inventory control and stock management
Basic microscope maintenance
Basic QC
Blood safety
Biosafety and waste management

[a] Giemsa stain is the recommended "gold standard", although some countries also use JSB or Field stains, particularly, in peripheral laboratories.

2.5.2 Quality assurance

QA will not be effective unless all the personnel involved are motivated and understand its principles and practices. Training in QA may be either separate or incorporated into training or assessment courses for malaria microscopy or supervisory visits. The main topics on which basic malaria microscopists should be trained for QA are listed in Table 2.

Table 2. Basic topics to be covered by training in QA for basic malaria microscopists

Topic
Consequences of deficient malaria laboratory services
Basic principles of laboratory QA and QC
Sources of errors in malaria microscopy
Essential elements of internal QC
Principles and practices of supervisory visits
Selection and dispatch of slides for blinded cross-checking
Principle and procedures of Giemsa stain QC
Procedure for cross-checking blood slides
Quality improvement (including corrective actions) in malaria microscopy
Effect on quality of equipment, reagents, stock control, workload, registration and reporting
Blood safety (including universal precautions)

Highly competent microscopists working at the national (central) and provincial (intermediate) levels will require more detailed training, particularly to acquire the necessary personal communication, teaching and technical skills required to supervise and improve the performance of laboratories and microscopists at peripheral levels.

2.6 Role of clinical staff in quality assurance

Appropriate ordering of testing by clinical staff also affects the operation of laboratory services. For malaria, clinicians should at least review the patient's recent clinical history, conduct a physical examination and act appropriately in cases of non-malaria febrile illness, including performing other basic laboratory tests, as indicated. Misuse of laboratory services by medical staff is a waste of scarce resources and leads to poor patient care.

The time required by a laboratory to give a clinician accurate results after blood film examination determines effective treatment and affects the confidence and satisfaction of patients with the health system. For malaria, the provision of results within 30–60 min is considered satisfactory. This goal requires both good laboratory services and effective collaboration between clinicians and laboratory personnel, working as a team with mutual benefit and respect. Improving laboratory quality can increase the confidence of both clinical staff and patients in the results of the blood film analysis.

Various practices can increase the confidence of clinicians in microscopy results:

- raising the awareness of health care providers and patients about the importance of blood film examination for a correct diagnosis;
- provision of training, reference reading material and guidance to clinicians on the clinical importance of microscopy examination and guidelines for requesting blood films in areas with different malaria prevalence;
- prominent display in testing centres of "competence certificates" awarded to resident microscopists;
- provision of personal log books certifying the competence of each microscopist;
- regular supervision and cross-checking of routinely prepared slides to confirm a continuing high standard of performance;
- participation in a proficiency testing scheme that includes malaria films, with certificates of performance displayed;
- joint supervisory visits by clinicians and laboratory technicians to health facilities, with feedback on performance and resolution of identified problems; and
- regular joint meetings between clinicians and laboratory staff to discuss issues and concerns.

3. PLAN OF ACTION

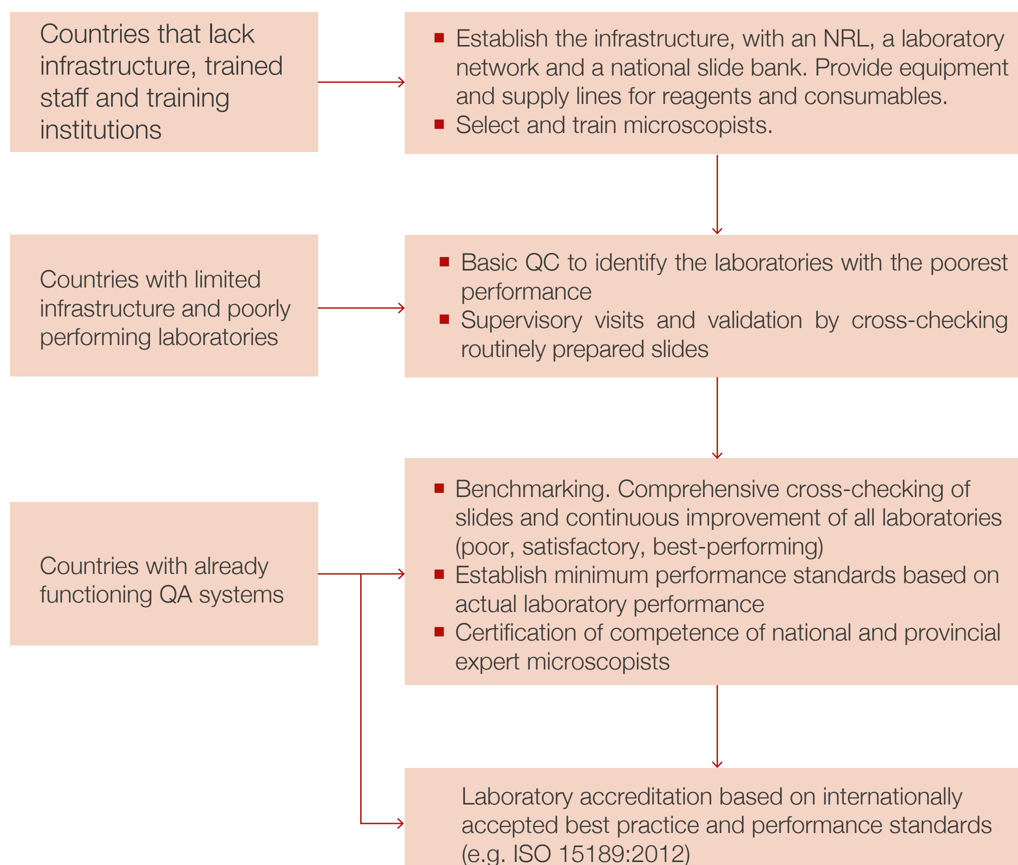
3.1 Goals and objectives

The long-term aim in all countries should be a fully functional national QA system, with benchmarking and certification of the competence of all microscopists. In order to assure such a system, QA programmes should prepare a national QA manual or guideline to:

- improve the overall competence and performance of microscopists at all levels of the laboratory service;
- sustain the greatest accuracy (both sensitivity and specificity) in confirming the presence of malaria parasites and identifying species;
- monitor laboratory procedures, reagents and equipment and the results of laboratory diagnoses systematically; and
- establish a clear hierarchical reporting system for the results of QA and feedback.

The time required to reach these goals will vary by country, as it depends on the baseline competence of microscopists, the resources available, the structure of the health system, the laboratory network and the incidence of disease. A model for progressive implementation of QA is outlined in Fig. 3.

Figure 3. Progressive implementation of QA in different contexts



3.2 Essential elements

National laboratory experts, clinicians and epidemiologists should interact continuously in preparing and implementing a plan of action and monitoring all activities for the QA of malaria microscopy.

The main elements of a plan of action for a laboratory QA system are:

- alignment with the priorities of the national laboratory services and the NMCP;
- a "gap analysis";
- the specific objectives and goals of the programme;
- expected outcomes;
- constraints that might affect achievement of the objectives and goals;
- activities to be conducted;
- a timetable;
- a detailed, realistic budget;
- a list of indicators for measuring the progress and outcomes of the programme, with appropriate reporting forms; and
- clear roles and responsibilities for key personnel.

3.3 Implementation

Effective QA should be conducted in a phased approach according to priorities. The colours in the illustration below indicate the order in which activities should be introduced to achieve a mature quality management system.

Core activities

1. Make a baseline situation analysis of the resources available in the country and gaps in commodities and infrastructure.
2. Identify the QA coordinator and a national core group of microscopists undergoing external competence assessment (ECA) and certified as WHO level 1 or 2.
3. Establish a national steering committee.
4. Ensure policies, guidelines, SOPs and associated commodities and infrastructure.

Second step

5. Competence assessment
6. Training
7. Supervision

Third step

8. Cross-checking
9. Proficiency testing
10. On-site evaluation
11. Accreditation of the diagnostic centre to international standards such as ISO 9001:2008, ISO 15189:2012 or ISO 17025:2005

The objectives of each national QA programme are adapted to the country context.

- In countries that lack the necessary infrastructure and adequately trained staff, it might not be possible to evaluate existing laboratory services, in which case priority should be given to refresher training of microscopists and building up the necessary infrastructure so that they can effectively perform their tasks.
- In countries with limited infrastructure and poorly performing laboratory services, the intermediate objectives should be to identify and improve the performance of laboratories and personnel and promote certification of national and regional microscopists.
- In countries that already have a functioning QA system, with trained personnel and some infrastructure, the objective should be to benchmark all laboratories to the highest standard, establish minimum performance standards based on actual laboratory performance and certify the competence of national and regional microscopists.

3.4 Situation analysis

The first step of the plan of action should be a situation analysis to determine the current status of QA in the country. The analysis should result in an accurate estimate of the resources required to ensure that QA can be implemented and sustained. The factors that determine effective implementation of a QA system are:

- the objectives of the malaria control programme and the role of parasitological confirmation of malaria;
- current organization of laboratory services for malaria diagnosis;
- the status or feasibility of integration with national laboratory services (depending on the objectives of the NMCP);
- the role and importance of the private sector and NGOs in malaria diagnosis and treatment;
- the existence and capacity of the NRL;
- the capacity of existing infrastructure and staff for training and for assessing the competence and performance of laboratory services;
- current availability of reagents and equipment;
- capacity of existing logistic systems to ensure provision of the necessary reagents and equipment and maintain the equipment in working order;
- the availability and use of guidelines and SOPs to ensure the quality of all aspects of malaria microscopy;
- reporting mechanisms; and
- current organization, status and performance of QA and current levels and sources of financial support for strengthening malaria diagnostic services.

Key issues to be considered in the situation analysis:

- Are the laboratories at each level appropriate for the work to be performed?
- Are there enough staff for the workload?
- Are the operating procedures up to date and followed by all staff?
- Are all staff adequately trained for the tasks they perform?
- Are the results produced acceptable, and do they meet the needs of the programme?
- Are suitable training materials and programmes available?
- Are the logistics for supplies of reagents and equipment adequate?
- Is there adequate budgetary provision for the tasks to be carried out?

The recommended steps for this situation analysis are shown in Table 3.

Table 3. Recommended steps for pre-implementation situation analysis

Task	Key issues	Notes
1. Make a chart of the laboratory network, showing relations and functions of different levels.	The network should be supervised by a NRL. Laboratories at the intermediate level should support peripheral laboratories.	When a formal network has not yet been established, a provincial or regional laboratory may support QA in peripheral laboratories as an interim measure.
2. Make an inventory of the available resources (staff, microscopes, equipment and budget)	Microscopists should have appropriate training in malaria microscopy. This will require an effective training and assessment programme designed for the needs at each level of the laboratory services. There must be an efficient system for the ordering and delivery of supplies and equipment. Each laboratory must have an electric binocular microscope with a x10 eyepiece and a x100 oil immersion objective in good working order (plus a x40 objective for non-malaria work); capacity for microscope maintenance is essential. The laboratory should have all the facilities for high-quality malaria microscopy examination. There should be regular communication between the laboratory, the clinical staff requesting a diagnosis and the NMCP. Laboratories should have appropriate administrative support. Refresher training and the frequency at which it is conducted should be considered, in addition to basic training.	Microscope performance is critical to providing a good-quality diagnostic service. Defective microscopes might not have to be replaced if effective maintenance and servicing are available. Electrical binocular microscopes are mandatory. Microscopy with direct light (sunlight) is not acceptable, as the resolution is suboptimal at low light intensity. If possible, the type of microscope used should be standardized throughout the laboratory services.
3. Collect data on the current workload, and assess the adequacy of resources with respect to the workload.	Staffing should be sufficient to provide effective, sustainable service (see section 3.5). Note whether staff receive incentives or compensation for their work and whether they consider it sufficient to ensure good service and/or their retention.	An excessive workload is a major contributor to poor performance.

Task	Key issues	Notes
4. Document all current QA activities, including QC. Collect data and evaluate performance. Identify limitations and causes of problems such as unsustainability.	The results of internal QA and slides for QC and performance in proficiency testing schemes should be forwarded to the intermediate or national level as required. QA should lead to improved performance. Details of corrective action should be documented.	The principles of QA should be included in all training programmes. QA should be part of everyday activities in all laboratories. Supervisory visits by adequately trained staff from the higher level of the laboratory service are essential for identifying and solving problems. They can improve staff motivation and programme performance. It is important to facilitate regular dialogue between supervisors and staff to ensure that the staff feel represented, recognized and free to voice their concerns or raise issues.
5. Assess the competence of microscopists at all levels of the programme.	National standards of competence should be established for each level of the QA system. Intermediate- and national-level microscopists should be trained and assessed for their capacity to evaluate basic laboratory operations.	The ultimate goal should be a cadre of highly competent microscopists certified according to international standards (e.g. WHO).
6. Determine the resources that are available and required for implementing or extending QA.	The goal is a national QA programme that comprises on-site evaluation, blinded cross-checking of slides and an effective proficiency testing scheme supported by an appropriate training and retraining programme and a logistics system to provide supplies and equipment.	

3.5 Workload

Excessive work is a major factor in poor performance. The sensitivity of diagnosis is directly related to the time available to examine blood films; it therefore decreases when the number of slides exceeds the work capacity of the microscopist. Even highly competent microscopists cannot perform at their best if they do not have the necessary time to correctly examine slides. The problem is compounded when microscopists also have the responsibility for diagnosing other diseases.

The WHO recommendation made during the eradication era, that a person can satisfactorily read 60–75 slides a day is now considered to be unrealistic, as microscopists today have different functions and roles in malaria control. It is now widely accepted that no more than 30–40 slides can be effectively read per day.

The time required to confirm the absence of parasitaemia (as in most cases of febrile illness likely to be selected for microscopy-based diagnosis) precludes such rapid turnover. Accurate counting of parasites, which is important in many situations in which

microscopy is used, takes a considerable time; and the time required to read positivity or negativity varies, as strongly positive thick films can be examined considerably more quickly than weakly positive or negative films.

Parasite prevalence varies and the work capacity of individual microscopists depends on factors including the quality of the microscope and the laboratory organization, the competence of the microscopist, the slide positivity rate and the parasite density. Thus, slide-reading capacity increases with more positive slides and higher average parasite densities. The reading time will be extended, however, if accurate quantification is required for clinical decision-making, even at high parasite densities. Another significant factor is the additional time required for species identification, when this is clinically important, which depends on whether the thick or the thin film is to be examined. Species identification from thin films at low parasite density is extremely time-consuming.

It is difficult, therefore, to recommend the number of slides that represents a reasonable workload in all situations. A guide to the minimum time required to examine a thick blood film for malaria parasites is given in Table 4.

Table 4. Estimated times for calculating the minimum total time required to examine a thick blood film for malaria parasites (assuming that the slide is of good quality)

Activity	Minimum time required
Locating and placing the slide on the microscope stage	5 s
Focusing x10, then adding oil and focusing the x100 objective	10 s
Microscopic examination of a high-density positive thick film to determine positivity or negativity	10 s
Microscopic examination of a low-density positive thick film to determine positivity or negativity	2–6 min
Microscopic examination of a negative thick film	6 min
Counting of the number of parasites/200 WBC in a positive film	10 min
Recording the result in a register	20 s

The actual time required for each step probably varies; however, the times given above approximate the reading capacity of a trained basic malaria microscopist. Very rapid examination of a slide with a high parasite density will give an indication of the presence of malaria parasites but does not allow reliable detection of the presence of a mixed infection.

The number of slides that can be examined also depends on whether the microscopist:

- performs only microscopy or has additional duties;
- only stains and examines the films; or
- performs all the functions necessary to obtain a microscope diagnosis (collecting blood from the patient, preparing and staining the blood films and examining them under a microscope).

An acceptable workload therefore depends on the context.

Table 5 shows the slide-reading capacity of a microscopist during a 4-h workday. Although microscopists may read for longer, 4 h of reading is likely to be typical, because:

- long hours of continuous reading result in fatigue, which can significantly reduce the accuracy of reading; and,

- in many clinics and hospitals, most patients arrive in the morning; microscopy is therefore concentrated in a peak period rather than being distributed throughout the working day.

Table 5 is based on the estimates in Table 4 that it takes 30 s to read a strongly positive slide and 6 min to read either a weakly positive or a negative slide and on the assumption that roughly half of all infections have a high parasite density and half a low density.

Table 5. Estimated maximum numbers of slides that can be examined in a 4-h workday (see Table 4), assuming no other duties, no involvement in blood film preparation, an equal proportion of high- and low-density slides and whether quantification of parasites is necessary

Slide positivity rate	10%	20%	30%	40%	50%
No counting					
Slides per hour	10	10.5	11.1	11.7	12.3
Slides per 4 h	40	42	44.4	46.8	49.2
Slides per 6 h	60	63	66.6	70.2	73.8
Counting					
Slides per hour	9	8.5	8.1	7.6	7.3
Slides per 4 h	36	34	32.4	30.4	29.2
Slides per 6 h	54	51	48.6	45.6	43.8

If the microscopist also collects and/or stains slides, the daily output will be significantly reduced. For example, if collection and staining requires 6 min, the time to examine a strongly positive slide will increase to 6.5 min and that for examination a weakly positive or negative slide to 12 min, thus reducing the average slide output significantly.

3.6 Costing of quality assurance programmes

The cost of implementing a national QA programme varies by countries for reasons such as:

- the goal, i.e. malaria control or elimination;
- the percentage of the population at risk of malaria;
- the status and effectiveness of the present system; and
- the country's implementation capacity, including the number of laboratories in the programme.

Preliminary studies suggest that the cost of implementation in countries with existing infrastructure and trained staff for QA will be relatively low. In countries that require scaling-up of QA, the short-term cost will be higher because equipment must be procured or refurbished and more human resources will be required to train and retrain microscopists and supervisors. The cost is largely driven by the number of facilities to be supervised and the travel and per diem costs of supervisors.

Countries may draw up programme budgets differently, but, whatever accounting system is used, the budget should be realistic and commensurate with the activities to be carried out. The essential components of costing the plan of action for QA implementation are listed below.

3.6.1 Initial costs

Central and intermediate levels:

- establishing the post of national malarial QA coordinator;
- national meeting(s) to prepare a QA strategy;
- collection for a national slide bank;
- nomination of a national reference group or NRL;
- conducting a situation analysis;
- training highly competent microscopists for supervisory roles; and
- purchasing slides and equipment.

Peripheral level:

- training basic microscopists,
- upgrading laboratories,
- procuring laboratory equipment and supplies and
- communication.

3.6.2 Recurrent costs

Central and intermediate levels:

- annual running costs: administration, including communication, equipment and supplies, staff travel and per diem; staff training and retraining;
- slide bank maintenance;
- slide validation, including inter-laboratory cross-checking, if feasible;
- supervisory visits;
- slide shipment and associated administrative costs; and
- annual meetings for programme review.

Peripheral level:

- laboratory supervision,
- annual replenishment of supplies,
- laboratory supplies and maintenance of equipment and
- administration and communication.

4. SUPPLIES AND EQUIPMENT

4.1 Standard lists

High-quality work depends directly on the quality of the equipment, reagents and other consumables. As the types and standards of equipment, reagents and consumables used in countries vary widely, many laboratories need guidance. Another challenge is operating an effective logistics system to maintain adequate supplies and ensure that equipment is in working order.

The NMCP is encouraged to prepare and endorse guidelines on the equipment, reagents and consumables required for malaria microscopy. They should include:

- a list of the minimum standards and specifications for equipment and supplies,
- recommendations for selecting microscopes and
- guidelines for assessing microscopes used in the field to ensure that they operate correctly.

The guidelines should take into consideration the contexts in which national programmes operate.

All the equipment and supplies procured and distributed to health facilities should meet nationally or internationally recognized standards. When this cannot be done immediately, standards should be set as soon as possible; Annex 1 of this *Manual* gives the minimum specifications for equipment, reagents and consumables. Standardization of microscopes (electric binocular microscopes are mandatory) will simplify both maintenance and the acquisition and supply of spare parts at country or sub-country level. Annex 1 also gives a model list of the supplies and equipment required for establishing a malaria diagnostic laboratory with a workload of 1000 slides over 3 months.

4.2 Establishment of a supply chain

The methods used to obtain reagents and supplies vary by country: some have a centralized, national procurement system, while in others laboratories can purchase the required reagents and supplies directly. An effective supply chain management system must be established in order to foresee needs and ensure the provision of all the equipment and supplies required for uninterrupted, reliable laboratory diagnostic services for malaria. An inventory management system should be created for equipment, including spare parts, reagents and supplies. Reagents and supplies should be replenished as required; however, if rapid replenishment of consumable items cannot be assured, buffer stocks for at least 3 months' operational requirements should be held at all levels.

Therefore, to ensure the uninterrupted availability of reagents and supplies and efficient, cost–effective laboratory operation, procedures should be in place for routine assessment of levels of consumption and of stocks of key reagents, supplies and spare parts for microscopes.

4.3 Microscopes

A reliable, well-maintained microscope is an essential requirement for accurate malaria microscopy. A binocular microscope with x10 eyepiece, an oil immersion lens (x100) and a built-in electrical light source is essential. The use of blue filters to increase resolution and change the light from that of ordinary electric bulbs to a more natural white light is also recommended. High-quality immersion oil with a refractive index of 1.5 should be used according to the manufacturer's recommendations. Alternatives, such as solar power, a mirror unit with a LED light or a light that shines directly into the microscope condenser are essential in areas where there is no electricity supply or there are frequent electric power interruptions.

To increase the life-span of microscopes, preventive maintenance, including cleaning the objectives and replacing parts as necessary, should be part of routine internal QC and must be properly recorded and documented. Microscopes should be covered when not in use to avoid exposure to dust, and proper precautions must be taken in humid areas to avoid fungal growth on the lenses and in the microscope.

4.4 Microscope slides

Only high-quality microscope slides, free of surface abrasions and purchased from a reputable supplier, should be used for malaria microscopy. Preferably, slides should have a frosted end for labelling; otherwise, a lead pencil should be used. The slides should be scrupulously free from grease, moisture or fungus and should therefore be cleaned and stored before use. This will prevent most of the artefacts that confuse a malaria diagnosis and will avoid detachment and washing away of thick blood films during staining.

It is recommended that slides not be re-used.

4.5 Staining reagents

Many differential stains have been developed for the detection of malaria parasites, but the Romanowsky stains, which stain the nucleus red and the cytoplasm blue, have proved the most adaptable and reliable for routine work.

The alcohol-based Giemsa stain is the "gold standard". It is the one most commonly used and the best for routine diagnosis because it can be used for both thick and thin blood films, is stable during storage and results in a constant, reproducible quality of staining at a range of temperatures. Although it is expensive, it is the stain of choice for diagnostic laboratories. Because of its importance in ensuring fine-quality staining, Giemsa stain powder should be bought from a reputable supplier, and stock solutions should be prepared in quality-controlled batches and distributed in-house to users. One of the critical variables in staining is the pH of both the staining solution and the water used for washing.

Simple hand-held pH meters should be available in all malaria diagnostic laboratories, as pH paper is not accurate enough for measuring the pH of water and buffers. Small differences in pH (such as between pH 7.0 and pH 7.2 or pH 6.5 and pH 7.0) can significantly affect stain quality.

Water-based Field and JSB stains are sometimes used, but they often give variable results, reducing their widespread application. These stains are not recommended for use.

4.6 Other supplies

High-quality microscopic diagnosis of malaria requires a continuous supply of other commodities including timers, markers, lancets, syringes, needles, Vacutainer-type needles, alcohol swabs, oil immersion lens-cleaning solution, lens-cleaning tissues, buffer tablets, pH calibration solutions, cotton-wool, gloves, safety glasses (including the over-spectacle type), filter paper and glycerol. Safety items such as gloves, sharps boxes, gowns and detergents, should always be available. In order to store standard slides for internal QC or to store patient slides for an external QA by a peripheral, intermediate or national programme, slide boxes should be available in any health facility that provides microscopic diagnosis of malaria.

Fuses and bulbs are relatively inexpensive and easy to replace. The availability of spare bulbs and fuses in a laboratory in which primarily microscopy is used for testing could determine whether a case is confirmed as malaria and should be a priority for procurement.

5. SELF-MONITORING OF LABORATORY PROCEDURES (INTERNAL QUALITY CONTROL)

5.1 Internal quality control

Internal QC is the daily control and monitoring of each stage of testing by laboratory personnel to ensure that all tests are performed accurately and precisely. Internal QC is a required technical competence of medical laboratories for quality and the quality management system. It is used to confirm or recognize the competence of medical laboratories by regulatory authorities and accreditation bodies.

Internal QC affects all the steps taken in routine laboratory procedures to ensure good-quality results. All laboratory staff should use it to check their performance and to ensure the reproducibility and sensitivity of laboratory diagnoses. The head of the laboratory is responsible for establishing internal QC in routine procedures, but all personnel must be involved and participate. A microscopist working in isolation should also routinely conduct internal QC, although the number of checks is more limited.

Internal QC is embedded in all laboratory procedures and is a continuous process. Its objective is to provide reliable results at all times, by:

- monitoring the accuracy and precision of the complete analytical process;
- immediately detecting and correcting any errors due to test-system failure, adverse environmental conditions or operator performance;
- monitoring the accuracy and precision of tests over time, which may be influenced by changes in test system performance, environmental conditions and operator performance; and
- rectifying any deficiencies.

5.2 Implementation

Procedures for internal QC should be initiated immediately in diagnostic centres. The initial level may quite basic, such as controlling staining, and developed step by step into a comprehensive programme for every laboratory.

The steps could be as follows:

1. Establish written policies and SOPs.
2. Assign responsibility for monitoring the policy and use of SOPs and updating them if necessary.
3. Train staff.
4. Obtain control materials.
5. Collect data.
6. Set target values and results.
7. Analyse and display control data regularly.
8. Establish and implement problem-solving and corrective protocols.

9. Establish and maintain a system for documentation.

Effective internal QC requires a "culture of quality" in laboratories, whereby staff understand the concept and use of internal QC.

5.2.1 Recommended routine activities

Each day. Stained QC slides should be used to check the quality and performance of the Giemsa stain. Malaria-positive blood should be used to prepare QC thick and thin films, which are then stored (for up to 2 weeks in a cool, dark, dry area) and stained at the same time as the next batch of patient slides. Before examining the stained patient slides, the QC slides are checked for the quality of red-cell staining to control the buffer quality, and WBCs are examined for staining of nuclei and granules and of parasite chromatin and red cell inclusions, if present. If the QC slides are satisfactory, the patient slides can be examined with confidence.

Each week. All staff should jointly review problematic slides encountered during the week, and a selection of slides from each microscopist should be rechecked by the head of laboratory or by cross-checking among staff.

Slides must be selected regularly for cross-checking, either by sending them to a cross-checking centre or during routine supportive supervisory visits. Cross-checking in the laboratory should be organized in a non-intimidating manner, by ad hoc structured, blinded checking of slides with unusual or uncertain aspects, followed by discussion between the validator and the microscopist. In most laboratories, both senior and junior microscopists should be involved, and all laboratory staff should work as a team. When an error is identified, the validator should review the slide with the microscopist, who should take corrective action, such as filtering or replacing poor-quality Giemsa stain.

Basic technical aspects that should be monitored regularly include:

- use of equipment, especially the microscope and its condition;
- the quality of reagents and stains, including storage conditions;
- the pH (7.2) of the buffer;
- accurate use of SOPs by laboratory staff;
- detection and recognition of parasites; and
- accurate completion of the laboratory register, logs, result work-sheets and internal QC records.

5.2.2 Addressing operational issues that affect quality

Additional operational issues that affect the quality and effect of malaria microscopy and that should be addressed include:

- timely reporting of results to clinical staff;
- coordination between clinicians and laboratory staff on diagnostic requirements;
- timely, regular submission of reports to the reference laboratory or other appropriate body;
- effective maintenance of supplies and equipment;
- readily available SOPs for all laboratory tasks, including those for internal QC, with strict control and training of staff in their use and regular (e.g. annual), formal, structured assurance and review.

Each testing laboratory in the network should adhere to internal QC procedures, with strict control of techniques and equipment as per national SOPs, to ensure:

- the reproducibility and sensitivity of parasite detection;
- periodic training and retraining of microscopists and other laboratory staff;

- the availability of well-functioning equipment;
- the availability of good quality stains and supplies;
- the quality of each prepared slide when examined under the microscope (when possible, any slide that is inadequately prepared or stained should be prepared again until a slide of an acceptable standard is produced);
- systematic compliance with the norms for internal QC by the coordinator of each malaria reference laboratory at national, regional and state level; and
- guides to resolve problems with equipment, reagents or methods for isolated laboratories where immediate help is not available.

The laboratory management should be aware of and monitor processes in which errors can arise, from slide preparation to examination and reporting of results, in order to reduce the possibility of errors. For microscopy, reference slides, bench aids, wall charts, etc., should be provided if required.

5.3 Corrective action

The main benefits of internal QC are early recognition of problems and swift corrective action, which must be taken whenever non-conformity is identified by internal QC. Technical processes must be available to make corrections, with effective means to prevent recurrence, such as adjusting the microscope stage, cleaning the objective, filtering or replacing stain and correctly storing stains and supplies. These actions are the basis of continuous quality improvement.

Internal QC procedures must be checked regularly during visits by technical staff from supervisory laboratories.

5.4 Measuring the impact of internal quality control

Indicators that can be used to measure the impact of internal QC include:

- laboratory registers or logs and internal QC records kept according to relevant SOPs;
- rates of corrective action;
- the reliability of laboratory results, whereby a clinician can establish a rapid, correct diagnosis;
- the reputation of the laboratory;
- the motivation of staff; and
- accreditation of laboratories.

6. EXTERNAL ASSESSMENT OF THE COMPETENCE OF NATIONAL CORE GROUP MICROSCOPISTS

The national reference group of microscopists must be highly proficient in all the tasks required, from preparation of blood films to accurate detection, counting and identification of parasite species and reporting and recording the results of the examination. This group, by definition, should consist of highly competent microscopists. To maintain their competence, they should be given:

- effective skill-based training;
- periodic assessment or monitoring of performance;
- supervision, with corrective action and re-training;
- reliable equipment and reagents; and
- an effective QA system.

The national core group must undergo regular assessment and certification of their competence to ensure that it is maintained. An external competence assessment of national core group microscopists is usually conducted by an external assessor who is a highly trained, competent microscopist skilled in assessments. A transparent system of assessment is necessary to ensure its credibility and authority.

The WHO regional offices for South-East Asia and the Western Pacific are preparing an SOP for the ECA programme in their regions. The plan is to extend the SOP to include the other WHO regions. ECA should be conducted under conditions similar to those of a good, operational malaria reference laboratory. This section refers only to certification of the national core group; other microscopists in the country should be assessed and certified in a national competence assessment (NCA) system (see section 7). The protocol for competence assessment and certification of peripheral-level microscopists in the national malaria microscopy programme should be appropriate to local needs (e.g. perhaps with less emphasis on the accuracy of parasite density determination and more emphasis on locally prevalent species); this might have to be preceded by an appropriate retraining programme. Certification of highly competent microscopists for clinical trials usually requires a more stringent assessment than the ECA.

ECA should be combined with some form of retraining, preferably after a test to identify the areas requiring revision. The proportion of time devoted to revision and the length of the courses will depend on the expertise of the participants. As leading microscopists, national core group members participating in an ECA should also be competent teachers of malaria microscopy and effective managers and supervisors in the national programme. For this, they might require training-of-trainers or training-of-facilitators courses, separate from the ECA.

6.1 Aims of certification

The purpose of assessment and certification of microscopists is to improve and sustain the quality of their capacity to diagnose malaria by standardized recognition of their skills. Specifically, certification of malaria microscopists should bring recognition of their achievements and career development. Furthermore, it can encourage others to aspire to a high skill level, to form a critical mass for the effective control of malaria.

It is strongly recommended that national programmes:

- formally recognize the skills of individual microscopists;
- design a training programme linked to certification, with appropriate recognition of the competence of microscopists at each level of the QA laboratory network;
- monitor competence continuously; and
- provide career paths for certified microscopists.

Recertification: It is recommended that certification be valid for not more than 3 years, with earlier reassessment for microscopists who are not performing well. If an earlier ECA is conducted, the results should be valid, irrespective of whether higher or lower competence is achieved.

Certification as a distinct activity: Certification of individual microscopists is separate from other forms of QA and cross-checking programmes, in which all microscopists and laboratories should be enrolled.

NMCP database of certified microscopists: The national QA programme should maintain a database of certified microscopists, including the national core group (levels 1–4) and other national microscopists (levels A–D) (see section 7.5).

Facility log of trained, certified microscopists: It is recommended that microscopists maintain individual logs of training and competence, with the relevant certificates and qualifications. One copy should be held and kept up to date by each microscopist and another by the laboratory supervisor.

6.2 Modality of certification

Competence should be assessed on the basis of the capacity of a microscopist to detect malaria parasites, identify species and quantify parasites accurately. Each participant must be informed, in a completely transparent manner, about their performance within the group and their resulting competence level. The examination results should be openly discussed and used constructively rather than punitively.

The assessment should set performance targets that are realistic, achievable and sustainable. They should include, at a minimum, grading of competence in parasite detection (presence), identification (stage and species identification) and parasite counting. Formal assessment and grading of blood film preparation and staining, microscope use and maintenance, biosafety and biosecurity and knowledge about malaria might also be included but should be graded separately from competence in microscopy. This additional assessment is important to ensure that a highly competent malaria microscopist is conversant with best practices in malaria microscopy, including good film preparation and staining.

6.3 Planning certification activities

Priority should be given to certification, which should be based on the number of highly competent microscopists required in the country and on funding. The certification programme should start with selection of participants who could work at the national (central) level, preferably including the NRL and academic institutions involved in malaria diagnosis and research. Regional and intermediate-level microscopists could be assessed once sufficient national microscopist have been certified as competent for their tasks in the national QA programme. Teachers of malaria microscopists should also be assessed to ensure that the pre-service training of microscopists is sufficient to build a critical mass of competent microscopists.

6.3.1 Objectives of the certification programme

The primary aim of certification is objective, formal assessment of the competence of malaria microscopists. A secondary objective is to provide refresher training, with standardized instruction and synoptic revision of the basic malaria microscopy curriculum. The ECA outlined in this *Manual* is designed to assess competence and to certify it on the basis of the assessment; it is not a training or retraining course. Experience has shown, however, that discussion of corrective issues during the assessment results in sharing of knowledge about ensuring good-quality microscopy. Discussion of the technical aspects of malaria microscopy in the ECA programme is a valuable form of revision.

Correction of minor technical mistakes in parasite detection, species identification and counting can rapidly increase the competence of microscopists, and the ECA should provide opportunities for such correction. Consequently, poorly performing microscopists receive information that will not only improve their skills but also motivate them to attain a higher level of competence when another ECA is conducted. The ECA should not, however, be a substitute for the training necessary to upgrade an average to a highly competent microscopist.

6.3.2 The ECA programme coordinator

A designated agency or institution with capacity for malaria microscopy can coordinate an ECA programme. The Asian Collaborative Training Network for Malaria (ACTMalaria) has played this role in the South-East Asian and Western Pacific regions, developing and managing the ECA programme and planning and managing the logistics of many country ECAs. Amref Health Africa has played a similar role in the WHO African Region. The two organizations are thus vital components of the success of the programme in Africa and Asia. Their current role is that of “coordinating agencies”. Similar agencies or institutions are required to lead the process in other regions and countries. The roles and responsibilities of coordinating agencies include:

- liaising and coordinating with the relevant WHO regional office, the NMCP and facilitators to prioritize and plan the calendar of ECA activities in the region;
- corresponding with the NMCP and securing government clearance for conducting the ECA;
- ensuring that the official list of participants is ready before the ECA and sent to the facilitators;
- overseeing promotional material, if needed, and ensuring that it makes appropriate reference to WHO and other partners;
- maintaining a register of facilitators and their competence in certification and issuing certificates (with unique numbers);

- coordinating training-of-trainer and refresher courses for ECA facilitators, jointly with WHO;
- participating in the selection and performance evaluation of lead facilitators, with WHO;
- liaising with WHO, the lead facilitators and other facilitators to ensure that the quality of ECA activities is maintained, specifically that:
  - the ECA complies with the approved WHO model;
  - facilitators are selected according to the recommended standards;
  - blood films from the relevant malaria slide bank comply with the WHO SOP;
  - certificates are awarded strictly according to the WHO model; and
  - the ECA complies with annual QA assessment of key performance indicators (see section 6.8); and
- maintaining a database of participants (with unique certification numbers) and results and reporting the results regularly to the respective WHO country office and region.

The coordinating agency analyses the information and sends a summary of relevant points to the hosting country and the WHO country and regional offices. The coordinating agency should consult programme managers and WHO country office focal points about progress being made. It is recommended that WHO analyse the results, give relevant feedback to the host country and follow up the programme.

Selection of in-country coordinating agency or institution should be based on its capacity to perform the roles and responsibilities listed above.

6.3.3 Facilitators

As the trainer or facilitator should have one-to-one interaction with the participants, the number of participants should be strictly limited, to e.g. a maximum of 12. The trainer must be highly experienced and have proven competence in malaria microscopy (usually certified as a WHO level 1) and be able to create a relaxed, respectful atmosphere. Each WHO region should establish a pool of such trainers.

Facilitators should be selected by the coordinating agency or institution and WHO, in consultation with the lead facilitator. An ECA facilitator must have the following qualifications or attributes:

- WHO-certified level-1 microscopist for the past 3 or more years;
- at least a graduate qualification in medical or laboratory science or allied health science;
- highly developed communication and presentation skills;
- extensive knowledge and experience in all aspects of malaria microscopy QA (only if QA is being assessed during the ECA);
- comprehensive knowledge of malaria parasite life cycles and malaria epidemiology; and
- experience in co-facilitating ECA with the lead facilitator and confirmation of suitability by WHO, the lead facilitator and the ECA programme coordinator, where relevant.

Desirable attributes for an ECA facilitator are:

- good competence, skill and experience in training and monitoring activities for malaria microscopists; and
- fluency in the WHO official language recommended for ECA by the WHO Regional Office.

The roles and responsibilities of the facilitator include:

- liaising with WHO and the coordinating agency or institution in planning and conducting ECA programmes;
- facilitating ECA and making the following reports:
  - draft report to the host country NMCP on completion of the ECA and to the WHO country office and
  - reports to the WHO regional office and the coordinating agency or institution;
- sending the completed evaluation forms to the coordinating agency;
- requesting blood films from the malaria slide bank that conform to the requirements of this *Manual*, examining the blood films, producing the test series required for the ECA and labelling the slides;
- making standard WHO presentations on all relevant aspects of malaria microscopy and QA covered in the ECA;
- producing a standard WHO theory test based on the 25-question model;
- producing all the documentation required to conduct the ECA:
  - timetable,
  - ECA evaluation sheet,
  - slide result sheets,
  - hard copies of the presentations and
  - theory test;
- undergoing ECA certification as required (at least once every 3 years) in order to to maintain level-1 status;
- attending facilitators' training courses as required to maintain good competence in training and facilitation;
- maintaining good competence in all aspects of QA for malaria microscopy; and
- complying with all other requirements of the SOPs in conducting the ECA.

The **lead facilitator** should be selected on the basis of performance in malaria microscopy and ability to teach and effectively facilitate ECA activities in all the cultural and professional situations in which the ECA are conducted. In addition to the skills and experience listed for a facilitator, the lead facilitator should also have a post-graduate qualification in QA.

The roles and responsibilities of the lead facilitator, in addition to those of the facilitator, include:

- being available to facilitators for advice and feedback on all aspects of facilitating the ECA;
- receiving summary reports of all courses;
- analysing trends in performance;
- preparing a concise annual report for the WHO Global Malaria Programme;
- ensuring that the standard and quality of ECAs is maintained, in conjunction with the WHO Global Malaria Programme and the coordinating agency, including maintaining the standard report and SOP formats and document-control templates; and
- as required, performing all the tasks of a facilitator.

6.3.4 Selection of ECA participants

The participants in national external competence assessments must be fully trained and be experienced practising microscopists. The selection should be based on an evaluation from objective questionnaires specially designed for potential high-level malaria microscopists. The assessment described in this section is designed for senior microscopists, who are expected to be reference clinical microscopists for slide cross-checking and training within the national malaria microscopy programme.

Table 6 is a template for a list of participants in an ECA, with the information requested from the host institute by the facilitator. The epidemiological data are collected and held by the ECA programme coordinator, if relevant.

Table 6. List of participants for external competence assessment

Name	Age	Sex	Designation	Education (highest level)	Place of assignment	Years in service	Details, duration and year of last malaria microscopy course attended

6.3.5 Preparation of participants

Before the ECA, the participants should be given the opportunity in their workplaces to revise relevant theoretical and practical knowledge and skills for malaria microscopic diagnosis. Rigorous preparation by participants before attending the certification course is a condition for a successful outcome. Anecdotal evidence suggests that microscopists who have had relevant retraining before the ECA achieve greater competence than those who do not. Participants should therefore be given a copy of the ECA curriculum and relevant SOPs at least 1 month before to allow time for preparation and revision. Other revision material, such as CD-ROMs of blood films or slide sets, should also be available.

Refresher training should include, at a minimum, understanding of:

- the life cycle of the malaria parasite;
- detection of malaria parasites, including at very low parasitaemia;
- identification of the four (five, if *P. knowlesi* is relevant) malaria parasite species;
- accurate counting of malaria parasites by the WHO-recommended method;
- preparation and staining of blood films;
- biosafety and biosecurity;
- correct use and maintenance of a microscope; and
- effective QA and QC.

6.3.6 Syllabus and timetable

The assessment should last a minimum of 5 days to allow sufficient interaction and assessment and improvement of the required skills. Clinical microscopists often find it difficult to absent themselves from their job for more than 5 days to attend a course; therefore, the curriculum must also include the capacity of an active diagnostic programme to operate in the absence of some laboratory staff.

The assessment should grade the participants on at least:

- microscopic detection of parasites on blood films and confirmation of parasite-negative films,
- correct species identification of parasites (the four major human malaria species) and
- Accurate counting.

Revision should include, at a minimum:

- detection, identification and quantification of human malaria parasites;
- correct use and maintenance of a microscope;
- relevant haematology (e.g. WBC, RBC, platelets);
- other parasites and pathogens in blood;
- artefacts on blood films; and
- effective QA and QC.

The programme should start with a test of theoretical and practical knowledge, with immediate feedback, and end with a blinded practical assessment on which certification should be based. A model syllabus and timetable for an assessment course for senior microscopists is given in Table 7.

Table 7. Model syllabus and timetable for an assessment course for senior microscopists[a]

Day 1, Monday [day and month]	
08:00–09:15	Registration, administration, ECA structure and expectations
09:15–09:35	Break
09:35–10:10	Test on theory and feedback
10:10–12:30	Practical test (10 slides)
12:30–13:30	Lunch
13:30–14:00	Microscope use and care
14:00–16:45	Microscopy practical test (8 slides)
Day 2, Tuesday [day and month]	
08:00–09:15	Review of practical test slides
09:15–09:35	Break
09:35–10:00	Parasite counting
10:00–12:30	Test slide examination (10 slides)
12:30–13:30	Lunch
13:30–14:15	Species revision
14:15–16:45	Test slide examination (9 slides)
Day 3, Wednesday [day and month]	
08:00–09:15	Review of test slides
09:15–09:35	Break
09:35–10:15	Parasite counting
10:15–12:30	Test slide examination (10 slides)
12:30–13:30	Lunch
13:30–14:15	Blood elements and artefacts
14:15–16:45	Test slide examination (9 slides)

Day 4, Thursday [day and month]	
08:00–09:15	Review of test slides
09:15–09:35	Break
09:35–10:15	QA in laboratory diagnosis of malaria
10:15–12:30	Test slide examination (10 slides)
12:30–13:30	Lunch
13:30–14:15	Training and revision options
14:15–16:45	Test slide examination (8 slides)
Day 5, Friday [day and month]	
08:00–09:15	Review of test slides
09:15–09:35	Break
09:35–10:00	Current and future diagnosis
10:00–10:30	ECA evaluation, certificates and closing

[a] This assessment is intended for senior microscopists who are expected to be reference clinical microscopists for slide cross-checking and training. The protocols for certifying lower-level microscopists and certification of microscopists involved in clinical trials should be modified for the requirements of those situations.

Each morning, the slides examined the previous day are reviewed openly and interactively, so that the participants can discuss or contest their results; this will contribute to learning and consolidation of the ECA. The results of all participants are openly displayed and discussed.

An ECA is an excellent opportunity to improve and strengthen the QA of the country's NMCP. If there is enough time, a limited assessment of the QA status of the country or organization might be conducted. The NRL or any other laboratory that functions as such could be visited and assessed against the criteria listed in Table 8. A checklist could be prepared for completion by programmes and organizations to capture the relevant information for planning certification. Table 8 briefly lists the questions that might be asked and the evidence that might be examined. The list is not exhaustive but gives a reasonable "snapshot" of current QA status.

Table 8. Checklist for national quality assurance

Item	Question	Evidence	Prompt or comment
Organization	Is there a diagram of the structure or a laboratory manual? Is there a central reference laboratory?	Confirm by questioning or a visit to the laboratory.	Authority? Resources? National core group of microscopists? Controlled training programme, QA project, ECA, cross-checking, supervisory visits?
Personnel	Does the laboratory have documented procedures for personnel management? Does the management maintain records for all personnel to determine their compliance with requirements?	Confirm by questioning. Confirm compliance by observing procedures, personnel files and training and competence records.	Current? Evidence of review? Document controlled?

Item	Question	Evidence	Prompt or comment
Equipment	Does the laboratory have documented procedures for selecting, purchasing and managing equipment?	Confirm by questioning and observation of documentation or an equipment management manual.	
Purchase and inventory	Are there documented procedures for selecting and purchasing services, equipment and reagents?	Confirm by questioning and examination of records, SOPs, equipment inventory cards and suppliers contacts.	
Process control	Does the laboratory have an internal QC system, a QA system, an external QA project or a proficiency testing system?	Confirm by questioning and examining records, QC records and proficiency testing records.	Results of external QA recorded and correct? Realistic turn-around time? Results reviewed? Errors investigated?
Information management	Does the laboratory have an effective system to manage information?	Confirm by questioning and examination of relevant records, standard test request forms, logs, work-sheets, relevant SOPs	
Documents and records	Does the laboratory have good-quality documents?	Confirm by questioning and examination of relevant records, SOPs for tests, quality manual, job aids, work instructions.	
Incident management	Does the laboratory have a system for reporting incidents?	Confirm by questioning and examination of relevant records, incident register, SOPs for incident investigation and corrective action.	
Audits	Does the laboratory conduct internal and external audits?	Confirm by questioning and examination of relevant records, internal and external audit reports.	
Process improvement	Does the laboratory monitor quality indicators?	Confirm by questioning and examination of relevant records, SOP for error investigation and corrective action.	
Customer service	Does the laboratory have a system for assessing and addressing customer satisfaction?	Confirm by questioning and examination of relevant records of customer satisfaction surveys, a suggestion register, SOP for complaint management.	
Facility safety	Is there a safety manual?	Confirm by questioning and examination of relevant records and the safety manual.	

6.3.7 Requirements for equipment and supplies

Before starting an ECA, the staff of the institute hosting the course must ensure sufficient numbers of good-quality, well-maintained microscopes, tally counters and calculators for the participants. A multi-head microscope is particularly useful and should be provided if possible. Consumables such as immersion oil and lens tissue are also required, and spare equipment, including back-up microscopes, microscope light bulbs and tally counters, should be available.

For instruction and assessment sessions and group discussions, the following items should be provided:

- 12 separate desks, with at least 1 m between participants;
- seats high enough (or adjustable) for microscope use;
- a large (preferably air-conditioned) room;
- a digital light projector;
- a screen for slide projection;
- power boards and extension cords;
- flip charts and a whiteboard with blue, red and black markers and an eraser;
- permanent markers (blue, red and black);
- an electronic timer; and
- name tags.

Participant should be given:

- a copy of the WHO *Basic malaria microscopy learner's guide*,
- a good-quality, well-maintained electric microscope with a x10 eyepiece,
- a notebook,
- black and red pens,
- two tally counters and
- a calculator.

Equipment and consumables to be made available:

- a computer with a printer,
- a back-up electricity generator,
- blue filters for the microscope if required (one for each participant),
- immersion oil (one container per participant),
- paper tissues (one box per participant),
- lens tissue (one pack per participant) and
- one or two spare microscope bulbs per microscope.

6.3.8 Malaria blood films

All four major human malaria species must be used during the ECA even if all the species are not usually present in the country or region, because national core group microscopists will probably be used for slide cross-checking and trainers of microscopists in other areas, where they might have to differentiate all species. Each slide must include a thick and a thin blood film. The parasites on most of the slides used for training and assessment must reflect the locally prevalent species, although the species incidence and parasite density may vary seasonally. The blood films must be of high quality and validated carefully before use. The species on all blood slides should be identified by polymerase chain reaction (PCR), and parasite densities must be validated by several highly competent (e.g. WHO-certified level 1) microscopists, who participate in ECA programmes; nevertheless, the assessment procedure and the course facilitator must be sufficiently competent and flexible to account for errors in previously validated slides.

The mandatory WHO ECA panel consists of three sets of slides:

Slide set 1 (42 slides) designed to assess microscopists' ability to detect the presence or absence of malaria parasites and identify the species on positive slides, consisting of 20 negative slides and 22 positive slides with low parasite density (80–200/µL):

- 10 *P. falciparum*,
- 4 slides with two species including *P. falciparum* (each species > 40 parasites/µL, co-infecting species according to local prevalence) and
- 8 *P. malariae*, *P. vivax*, and/or *P. ovale* slides (at least one of each species at a ratio according to local prevalence).

Time limit: 10 min per slide

Slide set 2 (14 *P. falciparum* malaria-positive slides) designed to assess microscopists' ability to accurately estimate parasite density:

- six with a parasite density of 200–500/µL,
- six with a parasite density of 500–2000/µL and
- two with a parasite density of 40 000–100 000/µL.

Time limit: 10 min per slide

Slide set 3 is used in the pre-course test on day 1. It contains a 30% subset of 18 slides from sets 1 and 2. For example;

- five negative slides from set 1;
- eight low-positive slides from set 1 (three *P. falciparum*, two *P. vivax*, one mixed *P. falciparum* and *P. vivax*, one *P. malariae*, one *P. ovale*); and
- five for counting from set 2 (two with 200–500 parasites/µL, two with 500–2000 parasites/µL and one with 40 000–100 000 parasites/µL).

Time limit: 10 min per slide

When possible, slides with unusual aspects, such as drug-affected parasites, poor staining, very high and very low parasite densities, combinations of mixed infections, artefacts and slides spiked with bacteria or fungi should be included for training and revision. For this purpose, an additional 10 slides with these characteristics should be included in the revision component of the course. Examination of these slides must not be included in the final assessment, as standardization between courses and countries is important.

6.3.9 Standard operating procedures

All malaria diagnostic programmes must have SOPs for basic microscopy maintenance, blood film preparation and staining. Any SOPs to be used in the ECA course should be provided to participants with the curriculum at least 1 month before the course.

6.4 Basic elements of the assessment

The competence of participants should be assessed both before and at the end of each course. The final assessment should be more extensive and form the basis for competence certification. The course should include revision of the basic elements of malaria microscopy and opportunities for one-to-one review of problematic films and discussion of errors of identification or parasite counting.

6.4.1 Conditions for assessment

A relaxed atmosphere is important, and participants should have time to familiarize themselves with the environment and equipment before the assessment. Strict examination conditions should be maintained to ensure silence and confidentiality. It is recommended that the assessment be spread over 3 days, as shown in the model timetable in Table 7.

High-quality electric binocular microscopes must be used and the participants allowed exactly 10 min to examine each assessment slide, which should be coded and given to participants in a random order. The code should be changed each day and the assessment spread over 3 days (if following the recommended WHO standard).

There should be no access to written or pictorial reference materials during the assessment, including the use of mobile phone data as it is not unreasonable to expect national core group microscopists to be able to detect, identify and accurately count malaria parasites without access to references.

Rather than giving each participant a box of 74 slides, it is recommended that one set be used. This will remove any perceived or real variation between sets of slides. The slides should be passed from one participant to the next in a serial fashion. This practice has a number of advantages.

- The participants examine the *same slide*, removing any (real or perceived) variation in species or numbers.
- If time allows, only 74 slides have to be pre-checked, rather than over 850 slides.
- It is easier to transport fewer slides, especially with baggage restrictions in air travel.
- It is easier to randomize and re-label 74 slides rather than 888.

The transfer of slides from one participant to the next can provide an opportunity to collude on results. To reduce collusion and any possibility of “cheating”, the process should be as follows:

- Ensure that slides are transferred by the facilitator and not participants.
- The facilitator counter-signs each participant’s response, using a distinctive pen colour, so that no further changes can be made on the answer sheet.
- Participants must not be allowed communicate during the assessment sessions. Each must have the necessary immersion oil, lens tissue, calculator, etc., and the facilitator should answer all questions.
- When two participants take a break, they are instructed not to leave the room together or to congregate anywhere they can communicate.
- To allow an equitable delay between receipt of the slides and the start of examination, slides should be collected from the microscopists sequentially in the same sequence in which they were delivered.

6.4.2 Pre-course assessment

Each participant is assessed before the course by a written theory test and a practical test of competence in examining a similar but smaller panel than that used for the final practical assessment. This will allow comparison of the results of the pre-test and the final assessment.

These initial assessments provided an indication of the baseline level of the theoretical knowledge and practical skills of the participant in malaria microscopy. The results are not used in calculating the participants’ competence.

6.4.3 Final assessment

While poor-quality blood films should be included for review and discussion during the course, the final assessment on which microscopists are graded should be with high-quality, well-validated blood slides. The assessment thus does not rely on the facilitator's judgement.

The recommended slide panel for the final assessment is described in paragraph 6.3.8. Each slide should include a thick film and a thin film. This panel is designed for assessment of the WHO-recommended competence certification standards.

6.5 Competence levels and certificates

The slide panel recommended by WHO is based on assessments conducted in Africa and Asia and was reviewed at WHO consultations in 2006 and 2008. The slide set panel and grading system are designed to ensure that chance (variation in the parasite distribution in a film) is highly unlikely to result in incorrect grading of the microscopist being assessed.

The WHO competence levels were set after many trials of the ECA model. There are four levels, level 1 being the highest and level 4 the lowest. To avoid confusion between the ECA programme and NCA programmes, the terminology used to grade microscopists should be different. For example, some NCA programmes use levels A, B, C and D (see section 7.5).

Microscopists are graded on the accuracy of species identification and of parasite counting. Parasite counts are scored as acceptable if they are within 25% of the true count (according to the validators used by malaria slide banks). The competence level achieved is based on whether the microscopist obtains the right results for parasite detection, parasite species identification *and* parasite counting. For example, to reach level 1 certification, a microscopist must achieve 90% or more accuracy for species detection and 90% or more accuracy for species identification, *and* 50% or more of the parasite counts must be within 25% of the true count (Table 9).

Table 9. WHO competence levels and criteria

Competence level	Parasite detection (%)	Species identification (%)	Parasite count within 25% of true count (%)
1	90–100	90–100	50–100
2	80–89	80–89	40–49
3	70–79	70–79	30–39
4	0–69	0–69	0–29

A recent change in the procedure for ascribing competence levels and certification is that only participants who achieve level 1 or 2 are certified as malaria microscopists at those levels. ECA participants who achieve level 3 or 4 are not certified but receive a certificate of participation, which state that level 3 or 4 was achieved; the level is also recorded in the facilitator's reports and in the ECA database.

The aim of ECA is to both assess and improve competence. The time devoted to assessment must be sufficient to ensure that microscopists with poor competence are not certified as sufficiently competent and that highly competent microscopists are appropriately recognized.

Certification programmes might extend the theory test by adding written and practical tests of blood film preparation and microscope maintenance. This will depend on programme needs and the time available; the course would have to be extended beyond 5 days.

The slide set and certification standards described in this section are designed for microscopists with important roles in national programmes, who have limited time to absent themselves from their normal duties.

For specialized microscopy, such as in drug or vaccine trials, the slide set could be extended to more thoroughly assess areas in which the results are highly sensitive, such as increasing the size of the negative slide set.

Certificates should show the competence level (1 or 2 for certification and 3 or 4 for certificates of participation) to allow comparisons between countries that issue certificates in local languages. Certificates should state the due date for the next assessment (a maximum of 3 years). The WHO certificate is signed by the respective WHO regional director and by the facilitator.

The certificate is awarded to participants on the final day of the ECA and is valid for 3 years from the date it was awarded.

6.6 Roles of microscopists after external competence assessment

Only microscopists certified at level 1 or 2 should train others, particularly at the national core group level. Level-3 microscopists could assist level-1 or -2 microscopists in training. Level-4 microscopists should not be involved in training, as they have achieved unacceptably low results for species identification or parasite counting, and careful consideration should be given to whether they should perform malaria testing without supervision and checking of results. Earlier retraining and certification should be required when poor competence is detected during cross-checking and supervision of the QA programme.

Certified microscopists must pass on their newly acquired knowledge and skills to their peers and to subordinate malaria microscopists.

6.7 Measuring the effectiveness of external competence assessment

The ECA programme should be assessed every 3 years at a joint review meeting involving WHO, the coordinator (when relevant), the NMCP and facilitators.

Participants should complete evaluation forms throughout the ECA and hand them to the facilitator on the last day. The facilitator will include the comments and suggestions (and responses) in the reports to WHO, the host organization or country and the coordinator, and WHO and the coordinator should use the feedback to improve the ECA programme.

Questionnaires can be sent from WHO or the coordinator to the host NMCP to assess the effectiveness of the ECA programme at national level.

WHO should analyse the evaluations of multiple ECA courses in countries and regions to gauge their effectiveness in improving the competence of malaria microscopists. This could be done by a "global lead facilitator".

The ECA could be assessed against the following key performance indicators:

- The lead facilitator complies with the requirements listed in paragraph 6.3.3.
- The facilitators comply with the requirements listed in paragraph 6.3.3.
- The blood films from the malaria slide bank comply with the WHO model, including the composition of the slide sets.
- The scoring used by the facilitator (cut-off points for grading specificity, sensitivity and parasite counting) complies with the WHO standard.
- Certificates are awarded according to the authorized cut-offs.
- Presentations comply with the WHO-authorized SOPs.
- WHO, the ECA programme coordinator, the NMCP and the facilitators comply with their terms of reference, as stated in this document.
- The participant evaluation forms are analysed and relevant changes made at the next ECA workshop.
- The responses obtained from the participants and the host NMCP are used to make subsequent ECA workshops more effective.
- The results of evaluations of multiple ECA courses in countries and regions are used to gauge the effectiveness of the ECA in improving the competence of malaria microscopists.

7. ESTABLISHING A NATIONAL COMPETENCE ASSESSMENT PROGRAMME

Malaria microscopists should be proficient in all the steps of preparing blood films, making an accurate diagnosis and reporting the results of malaria blood slides. Microscopists may be trained medical or laboratory personnel or, occasionally, other health workers trained in malaria microscopy.

Malaria microscopy must be accurate, for both patient safety and the credibility of the health service. Performance depends on several factors, the main one being the competence of the microscopist examining the slide. This is a specialized activity; it is relatively straightforward to recognize a malaria parasite, but a high level of skill is required to recognize parasites with a sufficient degree of reliability for use as the basis for managing suspected malaria – a potentially fatal but readily curable disease. To maintain the competence of microscopists, they require:

- effective training,
- monitoring of their performance and
- correction and retraining.

The competence of national malaria microscopists must be assessed and certified regularly by microscopists in the national core group, coordinated by the NRL.

The conditions in which the NCA is conducted should be those of a good, operational reference malaria laboratory. Peripheral-level (subnational) microscopists should be certified by highly competent national microscopists using a protocol appropriate to the local situation (e.g. identification of local malaria parasite species), with less emphasis on the accuracy of parasite density determination; it should perhaps be preceded by appropriate retraining.

NCA should be combined with some form of retraining, preferably after identification of areas of weakness. The time required for revision (the length of the course) will depend on the expertise of the participants.

National programmes should:

- identify a core group of national highly competent microscopists who can be recommended for an ECA on the basis of quantitative measures;
- give formal recognition to the skill of individual microscopists;
- develop a training programme linked to a system for certifying the competence of microscopists for work at each level of the QA laboratory network;
- monitor competence continuously; and
- provide opportunities for career advancement.

Certification should be valid for no more than 3 years, with earlier reassessment for those who wish to raise their certification level. Certification of individual microscopists is different from other forms of QA and cross-checking programmes, in which all microscopists and laboratories should be enrolled.

The national QA programme should maintain a database of course participants and their certification levels. Each microscopist should have a log book of training and competence courses, in which certificates and qualifications are recorded. One copy should be held and kept up to date by each microscopist and another by the laboratory supervisor.

7.1 Aims and principles

Assessment and certification of the competence of each microscopist can significantly improve the quality of malaria diagnosis and may further support their career development if it is linked to a defined career structure.

The primary aim of these courses is to assess the competence of malaria microscopists objectively and formally. An important secondary consideration is to provide refresher training, with standardized instruction and revision. The NCA programme outlined in this *Manual* is designed for assessment of competence and certification based on that assessment; it is not a training or retraining course. The national programme should provide opportunities for training, which may be linked to the NCA courses.

NCA should be based on the accuracy of detection of malaria parasites, species identification and parasite counting. Each participant must be informed, in a completely transparent manner, about their performance in the group and their resulting competence grading. The assessment results must be openly discussed and used constructively rather than punitively.

Assessments should be based on realistic, achievable, sustainable performance targets. They should include, at a minimum, grading of competence in parasite detection, parasite identification and parasite counting. Formal assessment and grading of blood film preparation and staining, microscope use and maintenance, blood safety and knowledge about malaria may be included but graded separately from microscopy competence.

7.2 Planning courses

The participants in a programme for microscopist certification should either already work or could work at national level; they should preferably include microscopists from the NRL. Lower-level or subnational level staff can be assessed once a sufficient number of national microscopists have been certified as competent for their tasks in the QA programme.

7.2.1 Coordinating institution or national reference laboratory

NCA courses are best organized and coordinated by a national or central reference laboratory, the roles and responsibilities of which are to:

- use national core group microscopists as facilitators;
- liaise and coordinate with the NMCP and plan the calendar of NCA activities;
- invite national, regional and provincial malaria programmes to nominate microscopists for the course (see selection criteria in section 6.3.3);
- coordinate with the NMCP for the preparation and signing of NCA certificates;
- participate in the selection and performance evaluation of facilitators in the national core group;

- liaise with facilitators to ensure that the quality and standard of NCA activities are maintained, specifically:
  - compliance of implementation with the approved model;
  - selection of facilitators according to the recommended criteria;
  - compliance of blood films from the relevant malaria slide bank with the requirements of the model;
  - awarding of certificates strictly in accordance with national standards;
  - compliance with a 3-yearly QA assessment of performance indicators; and
- maintain a database of participants and their results.

7.2.2 Facilitators

Facilitators should be selected from the national core group of highly competent microscopists. As one-to-one interaction between the facilitator and the participants is essential, the number of participants should be limited to, e.g. a maximum of 12.

The facilitator must be highly experienced, have proven competence (usually certified at WHO level 1 within the past 3 or more years), at least a graduate qualification in medical or allied health sciences, comprehensive knowledge of malaria parasite life cycles and malaria epidemiology and be able to create a relaxed, respectful atmosphere.

The coordinating facility or laboratory should identify a pool of facilitators.

7.2.3 Participants

Participants in NCA must be fully trained and be experienced practising microscopists. They may work in peripheral laboratories in areas of low prevalence or of malaria elimination, because ensuring the competence of the microscopists in those locations is critical to national malaria control and prevention. Microscopists must be selected on the basis of an objective evaluation made during national training or assessment courses.

Before the assessment, the participants should be given the opportunity in their workplaces to revise relevant theoretical and practical knowledge and skills for malaria microscopy diagnosis. Anecdotal evidence suggests that microscopists who complete relevant training before the NCA achieve greater competence than those who do not. They should thus be given a copy of the course curriculum and relevant SOPs some time before the course to allow time for preparation and revision. Other revision material, such as CD-ROMs of blood films or slide sets, should also be available.

Refresher training should include, at a minimum, understanding of:

- the life cycle of the malaria parasite;
- malaria microscopy
  - detection of malaria parasites;
  - identification of the four (five, if *P. knowlesi* is present) human malaria parasite species;
  - accurate determination of malaria parasite density by the WHO-recommended method;
- preparation and staining of blood films;
- biosafety;
- correct use and maintenance of a microscope; and
- effective QA and QC.

7.2.4 Syllabus and timetable

The course should last a maximum of 4 or 5 days to allow sufficient interaction and the assessment and improvement of the required skills. Clinical microscopists often find it difficult to absent themselves from their job for more than 5 days to attend a course; therefore, the curriculum must also include the capacity of an active diagnostic programme to operate in the absence of some laboratory staff. At a minimum, participants must be graded on:

- preparation of thick and thin blood films on the same slide;
- detection of parasites on blood films and confirmation of parasite-negative films;
- correct species identification of parasites (the four or five major human malaria parasites present in the country); and
- accurate counting.

Review sessions should include, at a minimum:

- detection, identification and quantification of human malaria parasites;
- correct use and maintenance of a microscope;
- relevant haematology (e.g. WBC, RBC, platelets);
- artefacts in blood films;
- communication and presentation skills; and
- effective QA and QC.

The course should start with a theoretical and a practical test (with immediate feedback) and end with a blinded practical assessment, on which certification should be based.

Each morning, the slides examined the previous day are reviewed openly and interactively, so that the participants can discuss or contest their results; this will contribute to learning and consolidation of the NCA. The results of all participants are openly displayed and discussed.

The structure of the NCA should be similar to that of the ECA course. The national programme should design a suitable course, such as a 4-day course with both learning and assessment modules. The learning modules might be didactic and focus on improving the baseline knowledge of participants. Day 1 could begin with an assessment of theoretical knowledge, blood film preparation and malaria microscopy. Days 2 and 3 would cover preparation of thick and thin blood films and parasite detection, species identification and parasite density determination. Competency levels should be announced and discussed on day 4. Group discussions on use of best practices once the participants are back in their laboratories could be held on the final day. An example of an NCA course structure is given in Table 10.

Table 10. Example of national competence assessment course structure

Day 1 Pre-assessment	Day 2 Assessment modules	Day 3 Assessment modules	Day 4 Assessment modules
Theory	Slide preparation (5 slides)	Slide preparation (5 slides)	Slide preparation (10 slides)
Slide preparation	Slide examination	Slide examination	Slide examination
Slide examination			Final discussion

7.2.5 Required equipment and supplies

Before the NCA begins, staff of the institute hosting the course must ensure that sufficient, good-quality equipment, consumables and reagents are available. A model list of essential equipment and supplies is given in section 6.3.7.

7.2.6 Malaria blood films

Slides of the four or five human malaria species should be used during the NCA, even if all the species are not usually present in the country, because microscopists may find non-local species in samples from travellers to the country. Furthermore, some participants may become validators and trainers of other microscopists who should be able to differentiate all species. Each slide should include a thick and thin blood film. The parasites on most of the slides used for training and assessment must reflect the locally prevalent species, although the species incidence and parasite density may vary seasonally. The blood films must be of high quality and validated carefully before use. The species on all blood slides should be identified by PCR, and parasite densities must be validated by several highly competent (e.g. WHO-certified level 1) microscopists who participate in ECA programmes; nevertheless, the assessment procedure and the course facilitator must be sufficiently competent and flexible to account for errors in previously validated slides.

The composition of the slide sets used for NCA should be similar to those used in ECA (see section 6.3.8).

When possible, slides with an unusual aspect, such as drug-affected parasites, poor staining, very high and very low parasite densities, combinations of mixed infections, artefacts and slides spiked with bacteria or fungi should be included for training and revision. For this purpose, an additional 10 slides with these characteristics should be included in the revision component of the course. Examination of these slides must not be included in the final assessment, as standardization between courses and countries is important.

7.3 Elements of the assessment

The competence of all participants should be assessed both before and at the end of each course. The final assessment should be more extensive and form the basis for competence certification. The course should include revision of the basic elements of malaria microscopy and opportunities for one-to-one review of problematic films and for checking errors of identification or quantification.

A relaxed atmosphere is important, and participants should have time to familiarize themselves with the environment and equipment before the assessment. Strict examination conditions should be maintained to ensure silence and confidentiality.

High-quality electric binocular microscopes must be used and the participants allowed exactly 10 min to examine each assessment slide, which should be coded and given to participants in a random order.

7.3.1 Pre-course assessment

Each participant is assessed before the course by a written theory test and a practical test of competence in examining a similar but smaller panel than that used for the final practical assessment. This will allow a comparison of the results of the pre-test and

the final assessment. These initial assessments provide an indication of the baseline theoretical knowledge and practical skills of the participants. The results are not used in calculating their competence.

Competence in thin and thick blood film preparation could be assessed for both individuals and the group. The instructor should note common errors made during this pre-assessment and correct them in specific learning modules. Course participants could also assess their own blood films to become familiar with the 12 quality standards that will be used to compute their final score. Each participant could prepare three blood slides with both a thick and a thin film and submit one of the three for grading. The next morning, participants would assess their own slide against the 12 quality standards before receiving their score from the facilitator.

Table 11 shows the scoring of blood film preparation. Each quality standard is worth one point, based on a "Yes" (1 point) or "No" (0 points) answer.

Table 11. Quality standards for grading blood film preparation

	Definition of quality standard	Yes	No
1	Is the blood film labelled correctly?		
2	Is the thick film of adequate size (about 10 mm in diameter) or 90% intact?		
3	Does the thick film appear to be of the correct thickness?		
4	Does the thin film appear without a feathered edge?		
5	Is there adequate space between the thin and the thick film?		
6	Does the thin film appear to have no spaces?		
7	Is the thin film ≥ 1.5 cm?		
8	Are the WBC in the thick film properly stained (is the nuclear material purple)?		
9	Are the RBCs in the thick film completely lysed?		
10	Do the RBCs in the thin film appear in a single layer towards the feathered edge?		
11	Do the RBCs show correct staining (is the colour grey–blue)?		
12	Do the thick and thin films contain debris, or is the background transparent?		

The facilitator can use the scoring mechanism shown in Table 12 for assessing the quality of malaria blood film preparation. For an objective measurement, slide preparation (quality of preparation and staining) should be graded on the criteria given below and converted to 100%. Each participant is expected to prepare 20 slides, with a maximum score of 5 points for each slide if it is graded "excellent". If all 20 slides are graded "excellent", the total score will be 100 points (100%).

Table 12. Criteria for assessing and scoring the quality of blood smears

Grade	Criteria for assessing the quality of malaria blood film preparation and staining characteristics	Score
Excellent	**Gross appearance:** Thin and thick film prepared on the same slide; thick film 10 mm in diameter; newsprint can be read under the thick film before staining 10 mm between the frosted end and the thick film and between the thick and the thin film, with a distinct head, body and tail.	5
	Microscopic appearance: RBCs lysed in the thick film and a monolayer of RBCs with normal and abnormal morphology in the thin film. Staining clearly distinguishes trophozoites, gametocytes, schizonts and WBCs from the background.	
Good	**Gross appearance:** Film with uneven tail, too thick, too wide or too long, of uneven thickness.	3
	Microscopic appearance: A monolayer of RBCs and fixed RBCs. Staining clearly distinguishes trophozoites, gametocytes and schizonts of malaria parasites and WBCs from the background.	
Poor	**Gross appearance:** Film with ragged tail, too thick, too wide or too long, of uneven thickness.	2
	Microscopic appearance: Distorted RBCs, malaria parasites and WBCs. Difficult to spot fields with a monolayer of cells.	

7.3.2 Final assessment

While poor-quality blood films should be included for review and discussion during the course, the final assessment on which microscopists are graded should be with high-quality, well-validated blood slides. The assessment thus does not rely on the facilitator's judgement.

Table 12 could also be used to calculate the final scores for malaria blood film preparation.

7.4 Competence levels and certificates

The competence levels and cut-off points should be decided by the NMCP. The slide sets recommended by WHO for ECA courses could be used to prepare national standards for the NCA courses, perhaps with lower cut-offs than for the current ECA standard.

The NCA could designate four competence levels, A being the highest and D the lowest, to differentiate them from the levels 1 to 4 used in ECA programmes.

Microscopists should be graded on the accuracy of species detection, species identification and parasite counting. Parasite counts could be scored as acceptable if they are within 25% of the true count. Microscopists could also be graded on their ability to prepare thick and thin blood films, as indicated above.

The competence level achieved depends on obtaining the correct results for parasite detection, species identification and parasite counting and also perhaps blood film preparation. For example, to gain level A certification, a microscopist would have to achieve 90% or greater accuracy for parasite detection and species identification, and 50% or more of their parasite counts would have to be within 25% of the true count. If

film preparation is included, the microscopist would have to achieve a score of 90% or more to attain level A. The lowest score among the four components determines the overall competence level.

The final assessment score is a combination of the results from two assessments: preparation of thick and thin blood films and malaria microscopy. The composite score is obtained by summing the results from days 2, 3 and 4, for parasite detection, species identification and parasite counting. The final score is converted into a percentage (Table 13).

Table 13. Basis for determining competence levels in a national competence assessment

Competence level	Parasite detection (%)	Species ientification (%)	Parasite count (within 25% of true count)	Preparation of thick and thin blood films
A	90–100	90–100	50–100	90–100
B	80–89	80–89	40–49	80–89
C	70–79	70–79	30–39	70–79
D	0–69	0–69	0–29	0–69

The slide sets used for NCA must be validated with the same stringency as those used for ECA. Otherwise, the scoring and certification of microscopists will be unreliable.

The NCA should not only assess competence but also improve it. The time devoted to assessment must be sufficient to ensure that microscopists with poor competence are not certified as sufficiently competent and that highly competent microscopists are appropriately recognized.

Certification programmes might decide to add written and practical tests of blood film preparation and microscope maintenance. This will depend on programme needs and the time available; the course would have to be extended beyond 5 days.

Certificates should include the grade (for example, levels A–D) to allow comparison among countries, which may issue certificates in local languages. Certificates should have the relevant logos, the dates of the course and the due date for the next assessment (a maximum of 3 years).

Each NCA certificate is signed by the NMCP and the facilitator and is awarded to the participant on the final day of the NCA.

7.5 Roles of microscopists after national competence assessment

The employment of certified microscopists should be decided by the NMCP, which might consider that only those certified at level A or B should train others, particularly at the national level. Level-C microscopists should assist in the organization of training only under the supervision of level-A or level-B microscopists. Those at level D should not be involved in training as they have achieved unacceptably poor results in species identification or parasite counting, and the NMCP should consider carefully whether should perform malaria testing without supervision and checking of results. Earlier retraining and certification might be required if poor competence is found on cross-checking and supervision in the QA programme.

Certified microscopists must pass on their newly acquired knowledge and skills to their peers and to subordinate malaria microscopists.

7.6 Measuring the effectiveness of national competence assessment

The NCA programme should be reviewed or undergo QA assessment (perhaps every 3 years) by the NMCP and the coordinating facility or laboratory. The assessment could include, for example, whether slide reading is 90–100% accurate on cross-checking, taking into consideration the possibility that other factors, such as better supervision, might improve the accuracy. NCA participants should complete an evaluation form and hand it to the facilitator on the final day of the course. The facilitator will include the comments and suggestions (and responses) in the reports to the coordinating facility or laboratory and the NMCP. This feedback should be used to improve the NCA programme.

The NMCP or coordinating facility or laboratory could send questionnaires to the diagnostic facilities of the participants to assess the effectiveness of the course; furthermore, they might analyse the information from a number of NCA courses to gauge their effectiveness in improving the competence of malaria microscopists.

The NCA could also be assessed on the basis of compliance with a number of performance indicators.

- The facilitator complies with the requirements listed in section 6.3.3.
- The blood films from the malaria slide bank comply with the NMCP model, including the composition of the slide sets.
- Scoring (weighting and cut-off points for grading specificity, sensitivity and parasite counting) complies with the NMCP model.
- Certificates are awarded according to the authorized cut-offs.
- Analysis of the participant evaluation form results in changes.
- The results from the questionnaires to the participants and diagnostic facilities are used effectively.
- Analysis of information from several NCA courses in the country or region is used to gauge the effectiveness of the NCA in improving the competence of malaria microscopists.

8. TRAINING OF MICROSCOPISTS

Skilled microscopists are vital to malaria programmes, and both diagnostic services and disease surveillance rely on their diagnostic and technical skills. Thus, training in malaria microscopy must be effective and must reach today's high standards. When microscopists are trained and can make quality-assured diagnoses of malaria, communities at risk for malaria have greater confidence in their services, clinicians use the microscopy services more effectively, and patients benefit.

Training and competence assessment are accepted as strategies to improve the quality of microscopy. Personnel at the forefront of malaria case management therefore require continuous updating and training. The level of competence to be attained by training depends on the type of training and the microscopy experience of the trainees. The required level might be different for ECA (see section 6), NCA (see section 7) and peripheral (clinic or village) microscopists. For example, achieving 80% accuracy in detecting malaria parasites (against a standard set of microscopy slides) may be considered achievable and satisfactory for a peripheral-level microscopist during training or competence assessment; trainers must indicate the standards they expect trainees to achieve. On completion of training, these microscopists will be responsible for diagnosing malaria on blood films from suspected cases in their communities, and important treatment decisions will be based on their competence. To gain the confidence of the public, clinicians and the health system, training must be of the highest possible standard.

Unfortunately, many microscopists have limited access to regular in-service training. This is a critical deficiency, as microscopists cannot be expected to give accurate results if they cannot maintain and improve their skills. Training must be comprehensive and cover all aspects of microscopy diagnosis of malaria, including theoretical and practical aspects. Comprehensive refresher training for a total of at least 2 weeks per year, perhaps in modules, is recommended. Training must include detection and recognition of all four or five species at different densities and in mixed infections, with emphasis on parasite counting by the WHO-recommended method (parasites/µL, against 200 or 500 WBC). The training slide set could also include drug-affected parasites and artefacts.

8.1 Objectives of training

Upon completion of the course, participants will be able to provide and facilitate good-quality malaria diagnostic services at all health facility levels, including at the peripheral level. They should have acquired the necessary skills and competence to:

- understand the epidemiology of malaria;
- understand and describe the importance of malaria as a potentially life-threatening disease;
- describe and demonstrate the precautions required when handling blood to prevent transmission of blood-borne pathogens;
- describe the common clinical signs and symptoms associated with malaria infection;
- accurately record patient details and results in the laboratory register;
- describe the biology of malaria parasites and vectors;
- prepare high-quality stains for malaria diagnosis;
- prepare thick and thin blood films, and stain them to a high standard;

- identify all malaria species (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*; and perhaps *P. knowlesi*) microscopically;
- identify all malaria parasite stages microscopically;
- differentiate artefacts and pseudo-parasites from true malaria parasites;
- identify other blood parasites;
- identify the components of normal blood;
- quantify malaria parasites accurately;
- maintain and store microscopes properly;
- prepare and maintain SOPs for malaria diagnosis;
- identify sources of error in malaria diagnosis and correct them; and
- correctly set up and maintain a microscope.

8.2 Selection of trainees

The trainees should be microscopists who work mainly in the malaria or parasitology section of a laboratory. After training, they will be expected to take a lead in establishing laboratory procedures, share their updated knowledge with other staff and supervise staff in their own facilities and others under their supervision.

The appropriate educational requirements of microscopists depend on a variety of factors. In many countries, only trained laboratory workers (minimum 2 years of training) perform malaria microscopy, while in others malaria microscopists are trained in specific short programmes. Experience has shown that workers with a wide range of educational backgrounds can be trained as malaria microscopists; however, if the entry level is relatively low, training might have to be longer: it will take longer to train someone with only 8 years of schooling than someone with 12 years.

Prospective trainee microscopists should:

- perform malaria microscopy diagnosis routinely;
- be able to read, comprehend and write in the local language;
- be able to follow a set of written instructions systematically; and
- have good hearing and eyesight.

In the past, colour-blindness was considered an exclusion factor for selection of malaria microscopists for training, but there is no evidence to support this restriction. Therefore, trainees should be given eye tests only if they experience difficulties before, during or after training.

Table 14 summarizes the selection criteria and the recommended length of training for microscopists

Table 14. Selection criteria and training requirements for malaria microscopists

Trainee	Selection criteria	Training
Person with no previous experience	Can read and write at a basic level If difficulties are found during training, test eyesight	Minimum 5 weeks at a level at least equal to the WHO training course Practical and theoretical examination
Laboratory technician	Experience in microscopy in a laboratory	Minimum 2-week training course Practical and theoretical examination

8.3 Method of training

8.3.1 WHO training manuals

It is recommended that training courses for microscopists are conducted in accordance with the principles and syllabus detailed in the WHO training manuals, *Basic malaria microscopy* part 1; *Learner's guide and basic malaria microscopy*, part 2; and the *Tutor's guide* (2010). The *Learner's guide* contains all the technical information needed by trainees in the field. The *Tutor's guide* gives extensive advice to those responsible for organizing, running and evaluating training programmes. The manuals are intended to standardize and facilitate teaching of all the tasks involved in malaria microscopy, outlining each step of the diagnostic routine in the sequence in which they should be performed in the laboratory. The style of writing and presentation of the manuals has been kept simple to avoid misunderstanding and to facilitate translation into other languages.

International biosafety guidelines (for example, *Biosafety in microbiological and biomedical laboratories*. United States Centers for Disease Control and Prevention, 2009) should be reviewed. Furthermore, WHO is finalizing a series of SOPs on malaria microscopy, including on biosafety and safe disposal of slides. These SOPs are not yet available; for further information, please contact Dr A. Bosman, Global Malaria Programme, World Health Organization, 20 Avenue Appia, 1211 Geneva, Switzerland. E-mail: bosmana@who.int.

The WHO *Tutor's guide* and *Learner's guide* may be used together (with audio-visual aids) for initial basic training and for in-service training. The *Learner's guide* may be used alone for refresher training or by individuals for reference.

Provision of the *Learner's guide* to trainees ensures that:

- all participants have the same training material, for standardized training and to avoid errors that can occur when trainees make their own notes;
- trainees can refer to the notes during training, facilitating their understanding; and
- after the course, all trainees can take home a set of notes that will be a helpful reference in their daily work.

Details of the organization of training courses for peripheral-level microscopists, including staff requirements, the syllabus, timetable and learning activities, are given in the WHO training manuals.

8.3.2 Assessing competence

The level of competence that the trainees have achieved in each of the components of the training course should be assessed objectively. The methods of evaluation and the expected grades of competence for basic microscopists are described below. Evaluation of the course by the trainees, the tutor and the facilitators will provide feedback to improve it.

Trainees should be monitored throughout the course and should not proceed from one learning unit to the next until the trainers consider that they have achieved the required practical competence, with particular attention to their ability to collect a blood sample, prepare thick and thin blood films, stain a blood film with Giemsa and correctly examine stained blood slides. The course provides trainees with information, opportunities for discussion and practical exercises to strengthen their competence in malaria diagnosis.

A basic assessment of competence should take into account the fact that many participants will have had only 5 weeks' experience (in the WHO basic course) when they take the examination. It is assumed that participants will rapidly improve their basic competence once they start applying the techniques in regular practice.

Upon completion of the course, microscopists, depending on their workplaces, will be expected to:

- participate in the management of malaria by ensuring malaria diagnoses,
- conduct QA and QC of malaria diagnosis in health facilities in districts throughout the country and
- facilitate support supervision and on-site training at all laboratory levels.

At the end of the course, each trainee should be assessed on their ability to distinguish between negative and positive slides, identify the parasite species and grade or calculate parasite density, according to their skill at the time of the examination. This should be based on a minimum slide set consisting of:

- 10 negative slides,
- 10 *P. falciparum* slides with a minimum density of 5 parasites/100 fields,
- 1 *P. vivax* or *P. ovale* slide with a minimum density of 100–200 parasites/µL,
- 1 *P. vivax* or *P. ovale* slide with a minimum density of > 100 000 parasites/µL,
- 1 *P. malariae* slide and
- 1 slide containing *P. falciparum* and either *P. vivax* or *P. ovale*.

Table 15 lists the minimum competence levels that should be achieved. Reassessment of a microscopist's competence by examination every 3–4 years is considered essential.

Table 15. Minimum competence levels for peripheral-level microscopists

Competence	Result
Sensitivity: Proportion of positive slides correctly read as positive	90%
Specificity: Proportion of negative slides correctly read as negative	80%
Accuracy of reporting *P. falciparum* when present	95%

8.3.3 Refresher training

Refresher training is considered essential for maintaining the competence and commitment of microscopists. It is recommended that:

- anyone performing malaria microscopy have refresher training every year,
- refresher courses should last a minimum of 1 week, and
- refresher courses should include more stringent training on species identification and, depending on the country, quantification.

8.3.4 Retraining

If a microscopist's performance is considered poor on the basis of slide cross-checking and proven to be due to incompetence during supervisory visits, the actions listed below should be taken.

- Additional supervisory and mentorship visits should be arranged for corrective training.
- The microscopist should be given two or three opportunities to improve performance.
- As appropriate, formal retraining should be provided (such as attending a further training course).
- The microscopist's eyesight should be checked.

If the microscopist fails to improve, he or she should not be permitted to examine and report on malaria slides.

8.3.5 E-training and e-assessment

E-learning is a mode of distance learning for developing skills and obtaining information by electronic means. Arguably, face-to-face learning is the most effective; however, it is not always possible due to financial, geographical or time restrictions. E-learning is not designed to replace traditional training and assessment methods but is a useful adjunct. Excellent providers of E-learning include Amref Health Africa and the CDC–WHO learning software CD, which can be accessed on the WHO Global Malaria Programme website (http://www.who.int/malaria/areas/diagnosis/microscopy_cd_rom/en/).

The benefits of e-learning include:

- no commuting or travelling to a venue;
- no additional travel costs to add to the budget;
- continuing to work while taking classes, thus allowing uninterrupted service, gaining work experience and immediately applying what is learnt from the course;
- no constraint due to the geographical location of trainees;
- learning new technologies and practising use of the Internet, office software, etc.;
- assessment of competence before attending a courses; and
- all students are equal: work is judged on merit alone, with no possibility for differential treatment due to race, gender, creed, sexual orientation, religion or disability.

The disadvantages of e-learning include:

- the requirement for access to a computer with reliable electricity and a good Internet connection;
- possible feeling of isolation or sense of detachment from the learning centre;
- unsupervised work, solving problems without support;
- requirement for self-motivation and discipline in order to progress through the programme in a timely manner; and
- no guidance or support during microscopy slide reading sessions, although some e-learning courses provide contact with a facilitator for specific queries.

8.4 Reporting

Comprehensive, effective training is an important component of an effective malaria microscopy QA system, and the outcomes must be reported regularly. When assessing QA, the availability of good training and assessment must also be checked during visits by technical staff from supervisory laboratories.

8.5 Corrective action

One of the main benefits of effective QA is early recognition of problems and swift corrective action. Corrective action must be taken when any non-conformity is identified in the training or assessment system. Deficiencies identified in the training programme should be corrected and effective mechanisms introduced to prevent their recurrence. This action will be the basis for continuous improvement of quality.

8.6 Measuring the impact of training

Indicators that can be used to measure the implementation and impact of training include:

- reports of participant satisfaction;
- evidence of an effective training programme (such as schedule and timetable);
- up-to-date records of training in the microscopist's folder;
- evidence that procedures are being performed correctly;
- better accuracy and reliability of laboratory results, thereby helping clinicians to establish the proper diagnosis rapidly, leading to better management of patients; and
- achievement of certification in NCA and ECA programmes.

9. OUTREACH TRAINING AND SUPPORTIVE SUPERVISION

9.1 Definition

OTSS is a decentralized method of supportive supervision by a team of clinical and laboratory supervisors whose competence has been assessed rigorously. They may function at national, intermediate, peripheral or even community level. Supervisory visits and on-site evaluations include a comprehensive assessment of the laboratory's organization, equipment, adequacy and storage of supplies, reagent quality, availability and use of SOPs, reporting of results, safety and infection control measures. On-site evaluation with a standardized supervisory checklist provides a realistic overview of malaria microscopy diagnostic services at the site, for supervising the programme, for correcting poor performance identified by cross-checking of slides and for providing strategies and corrective actions for immediate problem-solving.

The reasons for poor competence of microscopists include:

- inadequate training of graduates,
- no or little refresher training,
- limited, irregular supervision,
- inadequate and irregular QA (cross-checking and proficiency testing) and
- infrequent examination of blood films with the decreasing frequency of some parasite species in some regions.

Staff competence is only one of many factors that can affect performance. For example, most poor examination results are not directly due to the diagnostic ability of the microscopist but to:

- poor motivation or personal problems,
- a poorly maintained microscope,
- poor quality or incorrectly stored reagents,
- stock-outs of reagents or other essential items,
- poorly prepared blood films,
- poorly stained blood films,
- poorly labelled blood slides,
- excessive workload,
- reporting errors
- no updated reference documents such as SOPs and bench aids and
- lack of regular, sustainable funding for diagnosis.

OTSS can help fill some of these gaps. Each microscopist in a diagnostic facility can have face-to-face training, with reading of standardized blood films provided by the supervisor. It also offers an opportunity for cross-checking slide readings. Feedback on competence can be given immediately, providing an opportunity for focused training or revision. OTSS involves monitoring of performance throughout testing, including blood film preparation, staining and examination, to ensure good QA of malaria diagnosis by both microscopy and RDTs.

9.2 Objectives

OTSS involves mentoring, whereby an experienced, competent supervisor guides personnel in improving their microscopy skills. Its objectives are to:

- establish a trusting, respectful relationship between the supervisor and personnel that is conducive for learning,
- promote synergy between laboratory and clinical outputs,
- collect objective evidence of the status of testing and microscopy in order to take corrective action for continual improvement and
- provide regular (ideally quarterly) support to laboratory and clinical staff in facilities to promote teamwork, advocacy, monitoring and evaluation.

Although on-site evaluations are time-consuming and costly, they are essential to the operation of all QA programmes, as they enable the supervisor to:

- cross-check slides taken routinely;[1]
- correct errors in procedures on site;
- relate working conditions to the performance of staff assessed by independent cross-checking of slides;
- assess the internal QC and logistical procedures for maintaining equipment and supplies;
- ensure the availability of updated SOPs, bench aids and other reference material;
- identify any stock-out of supplies or reagents;
- discuss problems encountered by microscopists and laboratory managers, and suggest solutions;
- decide on training and retraining;
- build communication with staff in routine laboratories; and
- ensure retraining, if indicated.

9.3 Implementation

Slide cross-checking and on-site evaluation have distinct advantages and disadvantages and certain resource requirements. It is unlikely that any health facility will be able to implement all the methods fully without using a step-wise approach, taking into account the organizational structure, available resources and staff proficiency.

The following components are essential for establishing routine OTSS:

- involvement of policy-makers and management in planning and executing OTSS, with feedback to secure their commitment, financial support and authority;
- adequate human resources, including national or regional coordinators, competent supervisors and monitoring and evaluation staff to manage all aspects of the visit;
- regular training and competence monitoring of supervisors; and
- adequate funding for visits, feedback meetings and corrective action.

Experience suggests that joint laboratory and clinical OTSS promotes good communication and collaboration between the two. Joint supervision reinforces the role of the laboratory and of clinicians in the diagnostic cycle (pre-analysis, analysis and post-analysis). The goal is to ensure that accurate, valid malaria diagnosis, including microscopy and RDTs, is achieved every day. A standard checklist is used to identify problems that compromise the quality of malaria diagnostic and treatment services, and supervisors provide mentorship by on-site training and individual communication

[1] This will be essential in the early stages of a QA programme, when intermediate laboratories may be unable to undertake external QA due to inadequate resources.

with health workers. At the end of the supervisory visit, feedback is given to the facility managers on the findings and expected corrective actions, which should be reviewed at subsequent visits.

A detailed progress report for each facility, which is easily understood by both supervisors and health workers, should be used during the visit to target training where it is required. A final, approved report will be sent after the OTSS, which should be distributed to all responsible officers. The visits should be flexible, to include other health components and external QA schemes and to be modified to include all levels of the health care system. The visits should not be viewed as a one-time activity but rather as a means to make well-informed, evidence-based improvements in the quality of malaria diagnostic and treatment services over time.

The resource requirements and the steps involved in monitoring the performance of malaria microscopy laboratories are summarized in Tables 16 and 17.

Table 16. Requirements for monitoring the accuracy of laboratory or test centre results

Activity	Personnel	Administrative elements	Technical elements
Supervisory visits	Central or provincial staff trained in all aspects of QA for visits to intermediate laboratories Adequate numbers of intermediate-level staff trained in all aspects of QA for visits to peripheral laboratories Peripheral staff trained in principles and process of supervisory visits to smaller laboratories	Adequate funds to cover travel of staff from national or intermediate level to peripheral level Data analysis and reporting	Checklist of activities to be conducted during supervisory visit Standard monthly reporting form for use by peripheral laboratories
Cross-checking of blood slides	Adequate numbers of intermediate-level staff with skills required for rechecking and evaluating malaria blood slides submitted by peripheral laboratories, preparing feedback reports and conducting retraining	Sufficient storage for the number of slides required for cross-checking Reliable system for dispatching slides from a peripheral to an intermediate laboratory Effective communication system to report results from intermediate to peripheral laboratories	SOPs for blinded cross-checking of slides, including slide sampling, standard reporting forms and statistical basis of evaluations

Table 17. Steps in planning monitoring

Step	Assessment	Comments
1. Situation analysis		
2. Plan steps for establishing or improving monitoring methods, including a timetable for establishing minimal, intermediate and optimal activities.	What are the realistic short-term options for implementing or expanding monitoring? What methods best match the available resources? Which partners should be included in implementation and improvement? What is the priority of each action?	Consider the current level of performance, if known, and any existing monitoring activities. In the initial stages of establishing monitoring, little will be known about performance. Establishing a comprehensive country-wide cross-checking programme may take several years. Implementation should be step-wise.
3. Define and obtain the necessary resources.	Are additional resources available? What are the potential resources for obtaining additional staff, equipment and microscopes, and supplementary funds? What is the timetable for obtaining new resources? What data are required to support the need for additional resources?	Planning should ensure minimal time between identification of resource needs and their availability. Long-term planning may be necessary to obtain adequate resources to implement the monitoring programme fully at the optimal level. It is strongly recommended that SMART indicators be used in funding proposals.
4. Conduct pilot project, and document results.		
5. Evaluate and modify plan on the basis of the results of the pilot project.		The plan should be modified according to availability of resources. Particular attention should be given to the feasibility of the workload and to the validity of control measures.
6. Assess impact	Has corrective action resulted in better performance?	Improvement over time indicates that the methods are feasible and effective.
7. Modify and expand plan as required.		

OTSS supervisors must be trained regularly and their competence updated to ensure a high level of quality in supervision. Other monitoring mechanisms for supervisors might be used, e.g. national supervisors monitoring intermediate and peripheral supervisors.

9.4 Method

9.4.1 Human resources

Although policy-makers and other stakeholders are not involved in the day-to-day running of OTSS, they are important in planning and reviewing the programme. Routine training, assessment and monitoring of supervisors' mentoring skills and technical competence are required for effective OTSS visits. Therefore, supervisors should have the knowledge and confidence necessary to perform their duties. To use the results of OTSS visits effectively, supervisors must be able to interpret data to arrive at correct conclusions. They should use the results of OTSS not only to make informed decisions about the health facility and its staff but also to improve their own performance (e.g. filling in the checklist accurately and completely).

The extent of OTSS at the peripheral level will be determined mainly by the availability of human and financial resources. An investment should be made in identifying and assessing the skills of a pool of candidate supervisors during an NCA. Only the best-performing candidates with supervisory skills should be chosen. Thus, competent microscopists should be mentored to work as laboratory supervisors. This level of expertise may not exist below the national level and must be developed. In such cases, initial training, monitoring and even co-facilitation with more experienced supervisors should be conducted. While decentralization of OTSS visits is the goal, the time and resources required to develop this capability cannot be overstated.

National-level supervisors

The national level is responsible for training regional, intermediate and peripheral-level supervisors and for coordination with managers in the community. The cadre consists of senior technical staff who are highly competent in malaria diagnostics and case management and have extensive experience as trainers and supervisors. When possible, laboratory supervisors should be given the opportunity to link with international certification schemes, such as the ECA for malaria microscopy.

National supervisors oversee the quality of OTSS visits by conducting spot checks at health facilities. Because of their proximity to the health managers responsible for making decisions, they are often required to resolve complex issues that cannot be dealt with on site.

Regional, intermediate-level and peripheral-level supervisors

Supervisors at these levels are responsible for facilitating OTSS at the health facilities that provide malaria diagnosis and treatment services in their region. Their main role is to mentor health workers and monitor the quality of service over time. As for national supervisors, this level comprises senior technical staff with established competence in malaria diagnostics and case management. They may also have management roles; however, it is important that they have maintained good bench and clinical skills, as they will spend a significant amount of time during visits supporting technical areas such as malaria microscopy. Supervisors who have maintained competence in microscopy may be difficult to find owing to lack of opportunity and other commitments. Therefore, supervisors should participate in national in-service training and certification schemes and score at the highest level in order to be certified as highly competent.

OTSS coordinator

Coordinators are responsible for managing all aspects of OTSS at intermediate, regional or national level. They are the main point of contact for supervisors and provide both logistical and technical support during planning, implementation and after the visit.

Coordinators are also the liaison between regions and the national level. They are responsible for communicating results to programme managers and others and for programme advocacy. Coordinators also oversee data entry, quality and management. Coordinators work with other ministry of health units, such as monitoring and evaluation, to ensure that the data collected are used to make programme decisions.

Monitoring and evaluation staff

Monitoring and evaluation staff should be included in the development and monitoring of the OTSS programme. They are instrumental in preparing and revising OTSS checklists that are aligned with programme performance monitoring and plans. Ideally, the results of OTSS should feed into the overall health management system, which might include the health management information system or the district health information system.

9.4.2 Training of OTSS supervisors and maintenance of skills

OTSS training in mentorship and technical competence is generally conducted twice a year, although this frequency may not be feasible in some programmes. Separate curricula should be prepared for laboratory and clinical supervisors, but 2 days of joint sessions should be included to practise use of the checklist at a nearby health facility and plan OTSS visits. The curricula should include basic refresher training for malaria diagnostics, QA, QC and case management, with competence assessments in malaria microscopy for laboratory supervisors and fever investigation for clinical supervisors. The use of grading templates for competence assessments is encouraged to ensure standard, objective grading by facilitators.

A number of countries with functioning OTSS programmes have found that twice yearly or yearly training is insufficient to maintain a high level of competence in malaria microscopy. It is therefore recommended that microscopy skills be monitored routinely with proficiency-testing panels. Remedial training or even replacement should be foreseen for supervisors who fail to meet the minimum technical requirements.

9.4.3 Checklists for OTSS

A standard checklist is used during OTSS visits to track progress in achieving quality indicators and to monitor the effects of any training provided on site. The checklist should include a review of the findings at the previous visit, an inventory of capacity, observations, mentoring and recommended action.

The observations made on the checklist can give the supervisor an idea of routine practice and the basis for corrective action. Recommendations for corrective action should be made after the patient has been discharged, unless the error is considered dangerous for the patient. Prompts for supervisors to communicate or reinforce messages can be added to the checklist and changed according to the programme priorities. Some elements on the checklist can be collected annually or quarterly, such as power supply or slide rechecking, respectively, depending on the programme. The checklist should be revised annually on the basis of the supervisors' experience and data quality.

Although checklists will differ by programme, it is recommended that the following components be monitored routinely:

Laboratory components:

- level and number of laboratory staff;
- training of laboratory staff to diagnose malaria (within the past 12 months);
- water and power supply;

- microscopes, spare parts and maintenance;
- essential laboratory equipment;
- biosafety;
- stock-outs of essential supplies;
- reference materials (SOPs, bench aids, national guidelines and policies);
- procedures for internal QC;
- external QA by slide rechecking and proficiency testing;
- time for obtaining microscopy and RDT results; and
- reporting of test results.

Laboratory observations:

- Malaria microscopy:
- preparation of thick and thin blood films,
  - staining of thick and thin blood films,
  - examination of thick and thin blood films and
  - reporting results
- RDTs: preparation and reading of an RDT.

Clinical components:

- level and number of clinical staff,
- training of staff in malaria case management,
- clinical equipment,
- stock-outs of essential drugs,
- stock-outs of artemisinin-based combination therapy and other anti-malaria drugs,
- clinical documentation and
- reference material (national guidelines and policies, clinical algorithms and SOPs).

Clinical observations:

- preparation and reading of an RDT, when relevant;
- clinical investigation of febrile illness; and
- adherence to malaria test results in prescribing treatment.

The checklist is a monitoring tool and does not replace good follow-up in the form of discussions between the supervisor and health workers. While it is important to track progress in a health facility regularly, it is also important for the supervisor to mentor other health staff. Good supervisors will provide countless teaching moments in the form of corrective action, modification of a technique or sharing undocumented information. It is these types of interactions that form the backbone of the OTSS visit and improve both the safety and the quality of services at points of care.

9.4.4 The OTSS visit

An initial OTSS visit should take 2 days, depending on the size of the health facility, the number of staff, the number of supervisors per health facility and the extent of integration with other external QA schemes (e.g. proficiency testing) or disease programmes. Subsequent visits should take 1 day. Before the visit, the coordinator should manage all the arrangements, including printing checklists and transport and field allowances for supervisors. When possible, OTSS visits should be used to disseminate messages or distribute supplies from the national level.

Supervisors should use feedback or analysis from OTSS visits to modify their approaches for the next visit, such as changing their emphasis or identifying health staff who require more help. OTSS visits are dynamic, even though the checklist remains the same at each visit. This allows a targeted approach while alleviating the tedium of routine supervision.

9.4.5 Cost of an OTSS programme

Funding for OTSS visits should be secured at national or regional level. The budget should cover all the activities associated with OTSS, including training, monitoring, the visit and an annual workshop to review the programme. Programme costs will depend on the level of decentralization, the number of health facilities supported, the frequency of visits and the number of supervisors.

9.5 Monitoring and evaluation

9.5.1 Data collection

The basic elements of the system used to monitor and evaluate the quality and progress of OTSS should be both qualitative and quantitative and include:

- the national supervisory oversight mechanism (qualitative);
- the feedback mechanism between OTSS supervisors and coordinators (qualitative);
- the technical competence of OTSS supervisors (quantitative); and
- analysis, interpretation and dissemination of OTSS data (quantitative and qualitative) between supervising teams, health management teams and health facilities.

9.5.2 Reporting

OTSS produces not only data to be reported to national health information systems but also data for indicators of malaria case management. An ideal monitoring and evaluation plan includes indicators of progress and output for monitoring OTSS and a separate set of indicators for evaluating the results of OTSS for targeted health workers.

Progress and output indicators

- the number and percentage of OTSS supervisors who have been (re)trained in malaria microscopy, use of RDTs, clinical case management of febrile illnesses or OTSS practice and methods;
- the number and percentage of OTSS visits;
- the number of mentoring activities conducted and clearly linked to identifiable performance issues;
- the number of on-site training activities conducted and linked to identifiable performance issues;
- the number and percentage of bench aids provided as a result of OTSS visits; and
- the number and percentage of SOPs provided as a result of OTSS visits.

9.5.3 Measuring the impact of OTSS

The effectiveness of OTSS depends on the competence of supervisors, which should be monitored, with their performance. OTSS is a relatively new approach, and countries should learn from programmes in countries that have already used it. Techniques are being devised to assess a number of proposed outcome indicators:

- supervisor performance in malaria microscopy and RDTs;
- supervisor competence in identifying and rectifying errors in performing microscopy or RDTs;
- supervisor knowledge of clinical case management of febrile illness;
- health worker performance in conducting malaria microscopy and RDTs, including in facilities that meet quality standards (composite indicator);
- health worker adherence to national guidelines for diagnosis and treatment of malaria, with appropriate:
  - clinical consultation practice,
  - diagnostic measures,
  - diagnosis,
  - use of test results,
  - treatment practices and
  - patient counselling;
- routine, appropriate internal QA measures;
- stock-outs of essential malaria microscopy supplies, RDTs and essential drugs (including antimalarial drugs); and
- readiness of a facility to diagnose and treat patients with fever or malaria (composite indicator).

OTSS visits are dynamic; supervisors must exercise judgement in negotiating with the management and staff of the health facility about which deficiencies should be addressed before the next supervisory visit. The discussion must highlight those deficiencies that are most likely to affect the quality of diagnosis and treatment, those that are likely to be remedied or both. Although supportive supervision is costlier than training alone, Rowe et al.[2] suggested that supportive supervision can better increase worker performance.

[2] Rowe AK, de Savigny D, Lanata C, Victora CG. How can we achieve and maintain high-quality performance of health care workers in low-resource settings? Lancet 2005;366:1026–35.

10. CROSS-CHECKING MALARIA SLIDE RESULTS

10.1 Background and objective

Cross-checking is an important component of effective QA. It indicates whether a laboratory is providing accurate results and can detect major deficiencies in laboratory performance due to level of competence, poor equipment, poor reagents, poor infrastructure or poor work practices.

Cross-checking is essential in a malaria microscopy programme, although it should be seen as complementary to competence assessment, retraining, supervision and proficiency testing. Cross-checking may be done either at a cross-checking centre or at regular supervisory visits to the microscopists' workplace. Although programmes are encouraged to adapt OTSS to their context and requirements, many countries with limited resources cannot conduct OTSS initially and rely on cross-checking. Cross-checking must be coupled with a system for assessing and correcting poor performance, including retraining and addressing other factors that affect performance (see section 9.1).

Cross-checking large numbers of blood films tends to use significant personnel resources, and many malaria-affected countries lack the skilled capacity and resources to staff such programmes, resulting in many months of delay in cross-checking and little or no feedback to the microscopist being evaluated. This section recommends a method for sustainable cross-checking that has a high probability of detecting major deficiencies in performance.

10.2 Implementation and requirements

Countries that are initiating cross-checking should start with a few laboratories and check only slide preparation and staining. Once these aspects are mastered, with feedback, cross-checking of positivity rates can proceed.

Cross-checking of routine blood slides in a cross-checking centre demands human and financial resources and the following requirements.

- Both microscopists and their supervisors are adequately trained in the principles and operation of the cross-checking system.
- An efficient logistics network ensures the selection of slides at the periphery and their transport to the supervisory level for cross-checking or for storage if cross-checking is to be done by a visiting supervisor.
- Both microscopists and their supervisors are motivated and well-organized in operating the system.
- There is an adequate budget, and funds are available to operate and maintain the system.
- Microscopists send slides to the supervisory laboratory at the designated times and understand why they are sending them.
- There is good communication between the supervisor and technicians.

- The supervisor provides prompt feedback so that action can be taken to correct errors. (Late feedback loses impact and discourages microscopists.)
- The number of blood films to be cross-checked must be large enough to give a fair assessment of a microscopist's performance but small enough to be sustainable, not imposing too heavy a burden on the programme.

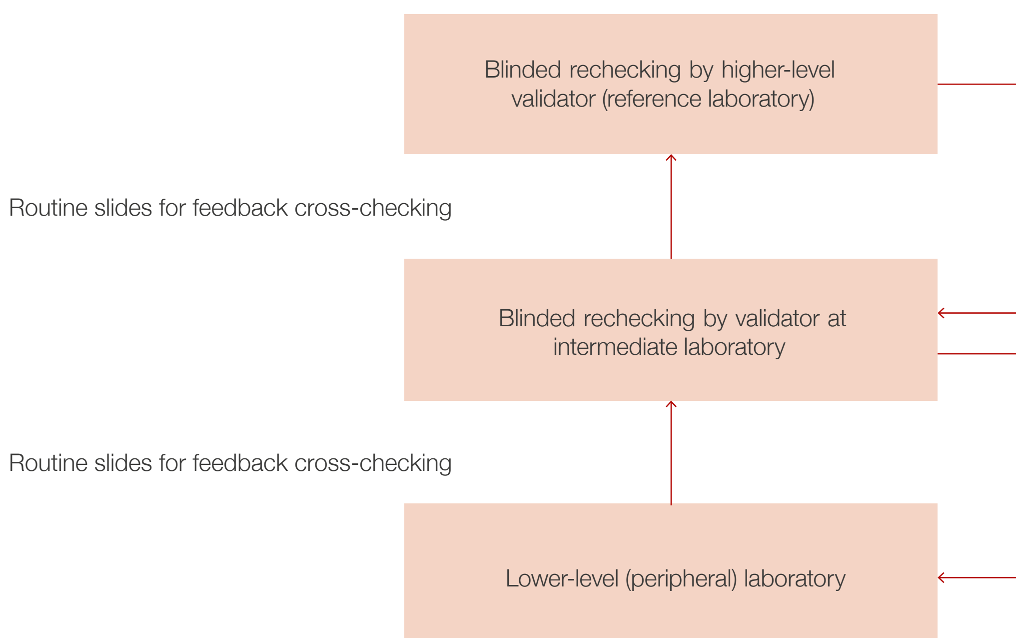
10.3 Principles and classification of errors

External QA by cross-checking is based on blinded re-examination of a selected sample of slides by staff at a higher-level laboratory. The validator undertaking re-examination must be highly skilled in malaria microscopy, have a thorough understanding of the sources of error and be able to compile the report that will be returned to the peripheral laboratory (and the NMCP). Re-checking must be done by certified malaria microscopist of proven competence. The microscopes used by the validators must be of good quality and in good condition.

Cross-checking also provides an opportunity to assess related elements of performance at the peripheral level. Blood films may be evaluated for specimen quality, appropriate size and thickness, and quality of staining. Problems detected by the validator should be noted on the report form, as this information may be useful for supervisors responsible for providing feedback to peripheral microscopists, determining the reasons for error rates and planning retraining and corrective action.

Ideally, microscopists should be cross-checked individually, but this may be impractical in laboratories with many microscopists. In such cases, the laboratory as a whole can be validated and individual microscopists validated internally by the head of the laboratory as required. The organization of validation is illustrated in Fig. 4.

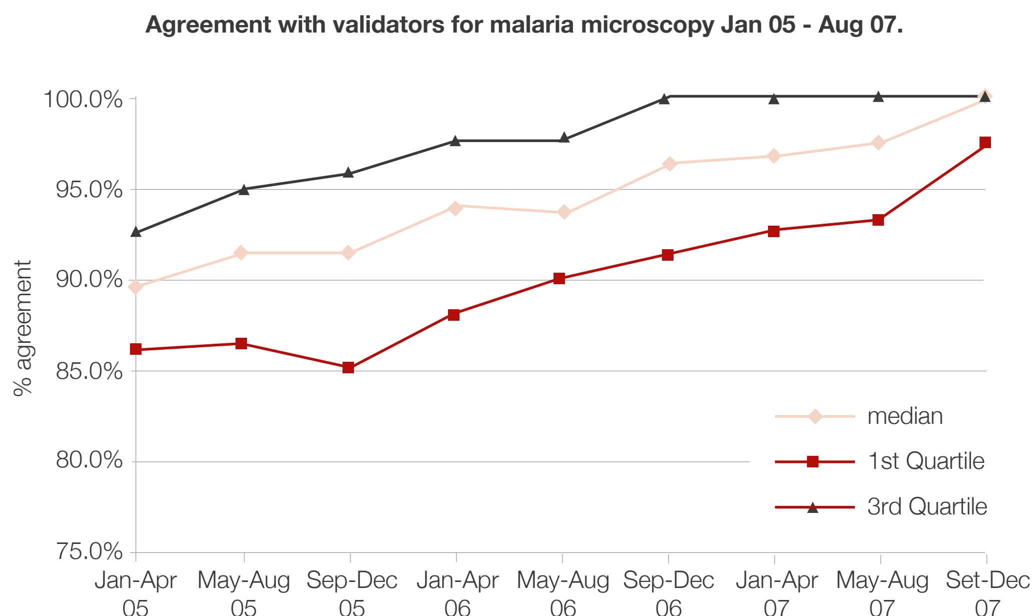
Figure 4. Organization of slide cross-checking



Cross-checking must be blinded to ensure objectivity; i.e. the validator who rechecks a slide must not know the initial result. Once a slide set has been examined and discrepancies are identified (differences between the clinical microscopist and the validators), the validator should recheck the discrepant slides with a further, un-blinded reading to confirm that there is no error, before reporting the result as discrepant.

The peripheral laboratory must be informed as soon as possible when a discrepancy is found between the reported result and that found by rechecking. The controlling laboratory should give feedback when appropriate, including probable explanations of the discrepancy and suggestions for corrective action. The results should be recorded in a database, which must be available to the supervisor before the next supervisory visit, at which discrepant results and the probable explanations must be discussed. Considerable improvement in laboratory performance is seen when constructive feedback and supervision are an integral part of cross-checking (Fig. 5). Common causes of errors detected at slide rechecking are listed in Table 18.

Figure 5. Improvement in laboratory performance after cross-checking in resource-limited settings, 2005–2007



From Klarkowski DB, Orozco JD. Microscopy quality control in Medecins Sans Frontieres programs in resource-limited settings. PLoS Med 2010;7:e1000206.

Table 18. Common causes of errors in blinded slide rechecking

Possible causes	Notes	Suggested actions
Initial laboratory true positive – cross-check false negative		
Very low parasite density	There is a 34.8% probability of a false-negative result at an average parasite density of 1 per 100 fields. As the validator should perform a second examination if there is a discrepancy with the initial reported result, he or she will read at least 200 fields. Even then, there is a 10.7% chance that 1 parasite/100 fields may not be detected.[a] As the QC protocol requires selection of slides with a low parasite density for cross-checking, this problem may recur often.	
Stain faded since original examination	Can be minimized by use of high-quality stains in laboratories, ensuring that cross-checking is done with minimum delay, and storing slides in cool, dry places	Re-stain “false negatives”. The validator should assess the staining quality of other components (such as platelets) to assess staining quality. Re-staining is usually appropriate only if the stain is likely to have faded.
Too high a QC workload for the validator	One of the principal reasons for the small sample size in the QC protocol is to reduce the workload of reference laboratories and other validators.	Reduce the workload of the validator (perhaps increase the number of validators).
Poor skill level of validator	The QC protocol is based on the premise that cross-checking is performed to a high standard.	A major problem. Consider training or changing the validator
Pressure on laboratory staff to find malaria parasites when there is a clinical suspicion of malaria	Some clinicians may criticize laboratories (and assume poor-quality slide examination) that report negative findings for patients with symptoms consistent with malaria. As clinicians have a higher status than laboratory staff, such pressure can be very strong.	Clinicians should be fully aware of laboratory QC results: if the QC results are good, clinicians should have confidence in the results.
Initial laboratory true positive – cross-check false positive		

Possible causes	Notes	Suggested actions
Laboratory staff report negative slides as “weakly positive” because they consider this “safer”.	A major problem due to either lack of skill or confidence or pressure from clinicians	Retraining to increase the skill and confidence of laboratory staff. Assessment of the programme to identify any external causes of the problem (such as pressure from clinicians to report positive findings)
Artefacts such as stain deposit or unfiltered water incorrectly interpreted as malaria parasites	Common causes are staining with dilute Giemsa stain > 15 min after preparation; using tap water to prepare buffer; using poorly cleaned slides; fungus-contaminated slides	Prepare diluted Giemsa stain immediately before use. Always use filtered or bottled water for preparing the buffer. Use only new slides or slides that have been fully cleaned. Never use slides that have been contaminated with fungus. Retraining of staff in good laboratory technique and recognition of artefacts
Howell-Jolly bodies and platelets misidentified as malaria parasites	Due to poor slide examination skill. Platelets are less of a problem, as laboratory staff are familiar with their morphology; they may be less familiar with Heinz bodies.	Retraining
Poor skill levels of laboratory staff	All of the above	Retraining
Clerical error	Can occur at any time. The “clerical error index” allows assessment of the overall rate of clerical errors in a laboratory; however, occasional clerical errors can occur even in laboratories with an overall high standard of clerical accuracy.	Depend on the clerical error index. If this is unsatisfactory, the laboratory clerical systems should be reviewed and improved. If the index is satisfactory, no action is indicated.
Initial laboratory true negative – Cross-check false negative		
High workload, so that microscopists examine slides too quickly	The maximum workload capacity (number of slides/h) of a microscopist should not be exceeded. Malaria slides are often examined in a laboratory during a peak period rather than regularly throughout the day. The laboratory workload capacity must be managed particularly during these peak workload periods. The workload capacity of a laboratory may be limited by a shortage of both microscopes and staff. For example, if there are six laboratory staff but only one microscope, the workload must be calculated on the basis of only one reader.	Workload management

Possible causes	Notes	Suggested actions
Poor skill level of laboratory staff		Retraining
Poor staining technique	If a good-quality stain is used, poor staining can be attributed to staining technique,	Retraining in staining methods. Ensure that the following are correct: ▪ buffer pH, ▪ Giemsa diluted immediately before use and ▪ staining time
Clerical error	All laboratories must have stringent protocols to minimize clerical errors.	Review pre- and post-analytical protocols, and ensure that they are respected.
Poor skill level of the validator	The QC protocol is based on the premise that cross-checking is performed to a high standard.	A major problem. Consider training or changing the validator.

[a] Raghavan K. Statistical considerations in the microscopical diagnosis of malaria, with special reference to the role of cross-checking. Bull World Health Organ 1966;34:788–91.

10.4 Method and protocol for slide cross-checking

The method and protocol are based on:

- minimal sample selection,
- selection of weakly positive slides,
- accurate cross-checking,
- rapid availability of QC results,
- valid statistical analysis of results and
- central reporting and analysis of results (benchmarking).

10.4.1 Objectives and scope

The overall objectives of this protocol are to:

- provide a standard method for malaria microscopy that is simple to use, easy to understand and requires a minimum QC sample size;
- form the basis for realistic minimum standards based on actual performance;
- enable benchmarking; and
- promote a high standard of laboratory testing.

The protocol is applicable for laboratories and test centres for routine diagnostic malaria microscopy. The small sample size is not applicable for QC of blood slides taken for research purposes, such as clinical trials of new drugs and vaccines or monitoring resistance to antimalarial drugs.

10.4.3 Slide storage

All routine slides examined by a laboratory must be stored in secure slide boxes protected from excessive heat and humidity until the QC slides have been selected. Slides must be stored consecutively according to the laboratory identification number, so that there is a direct link between the results in the laboratory register and the slide location written on the slide box, according to the date on which they were processed. Slides can, however, be stored in the most convenient way for the individual laboratory, either divided into positive and negative or sequentially.

The stored slides should be free of immersion oil, and the laboratory number should be clearly visible; the results of examination of the blood film should not be written on the slide. Routinely prepared slides must not be discarded until the QC slides have been selected.

10.4.4 Sample selection from the laboratory register

As QC depends on correct selection of the sample, the sample selection protocol for each test must be followed scrupulously. The three critical determinants are the method of selection (random or systematic, with no opportunity for selection bias), the minimum sample size and the selection criteria. The QC sample must be selected from the laboratory register. Microscopy slides for cross-checking must not be selected directly from slide storage boxes.

When the number of tests performed is less than the minimum sample size, all slides must be cross-checked.

The laboratory supervisor is responsible for randomly selecting a minimum of 10 slides each month (five reported as low-density, five reported as negative) for QC, using a random numbering system. If a random numbering system cannot be generated, selection should be based on random or systematic sampling independent of the microscopist(s) being checked. It is important that QC slides be selected randomly from routine tests performed during the calendar month or more recently (see below). Therefore, routinely prepared slides must not be discarded until the QC slides have been selected.

Five weakly positive slides with a parasitaemia of 20–200 trophozoites/µL and five negative slides should be selected. Slides with parasite densities > 200 trophozoites/µL should **not** be selected.

This protocol results in preferential cross-checking of weakly positive thick blood films in order to maximize the statistical power. Cross-checking of strongly positive blood films provides minimal information because:

- even poorly skilled microscopists can usually detect strongly positive slides, reducing their usefulness for monitoring sensitivity; and
- the probability that a strongly positive blood film will be reported as a negative is extremely low (except in the case of clerical error).

Strongly positive blood films often represent a significant majority of the positive slides examined in laboratories. A random selection of all positive slides will therefore include insufficient weakly positive slides to give a meaningful measure of performance. (For example, if 60% of blood films in a programme are strongly positive, a random selection of five positive slides is likely to include two or fewer weakly positive slides. This is too few to be statistically significant.)

To avoid selection bias, a clear selection protocol must be established in the SOPs, based on a random selection from a list of all low-density positive slides and all negative slides.

In laboratories with a high workload, where a minimum of four or five weakly positive slides will be sent each week, weekly QC sampling may be done according to the following strategy:

- week 1: randomly select two weak positives and one negative slide
- week 2: randomly select one weak positive and two negative slides
- week 3: randomly select two weak positives and one negative slide
- week 4: randomly select one weak positive and two negative slides.

If transport is problematic, slides may be collected weekly, grouped and dispatched monthly to the cross-checking laboratory.

10.4.5 Cross-checking

All 10 slides should be cross-checked for the presence or absence of parasite stages and for the accuracy of differentiation of *P. falciparum* and non-*P. falciparum* parasites. Cross-checking of five weakly positive slides per month (i.e. 20 slides in a 4-month period) limits the statistical validity of species identification; nevertheless, it is better thoroughly to cross-check 10 slides than to cross-check a larger number inadequately. Laboratories are encouraged to perform more QC than the minimum requirement, **provided** that there is sufficient capacity for all QC slides to be cross-checked accurately.

Timing

Cross-checking should be done as soon as possible after the end of each month and the results reported optimally within 2 weeks. Unfortunately, this is not usually feasible. When, for practical reasons, it is not possible to meet this delay, a longer time may be acceptable; this must be for reasons of necessity and not convenience and **must** be approved by the QC supervisor. An important principle of the QC protocol is that the results are an integral part of laboratory management and must be available and analysed as soon as possible.

Selection of cross-checker (validator)

QC depends on accurate cross-checking of QC slides. Validators or cross-checkers must have proven competence (e.g. WHO-certified level 1 or 2); if they have the necessary competence, they could be selected from among the following personnel (in order of preference):

- suitably trained members of the NRL,

- similarly competent members of the regional laboratory or
- the laboratory supervisor or an experienced laboratory staff member appointed by the laboratory supervisor (internal cross-checking).

The competence of all must have been assessed and found to be to the required standard within the previous 3 years. The validators must then be enrolled in an external QA programme with some form of internal or external cross-checking.

When QC cross-checking is performed internally, the identification of the slides must be concealed and all slides identified only by an internal QC number. The person cross-checking the slides must be blinded to the identity of the slides. The person who selects the QC slides should not be the same person who cross-checks them.

Accuracy of cross-checking

Slides must be cross-checked with considerable care. The accuracy of cross-checking is expected to be higher than that of routine slide-reading; therefore, cross-checking may reveal weak positives that were undetected in routine work. This should not be considered an error by the person who performed the routine examination, unless that person repeatedly reports false-negative results. Low sensitivity in routine examination is frequently due to variables such as high workload and poor equipment and not to lack of skill of the reader. Cross-checking is intended to approach a gold standard level as closely as possible.

10.4.6 Recording results

All results should be recorded in a 2 x 2 table, as follows:

(i) QC monitoring based on identification of asexual blood parasite stages but without species identification:

Routine laboratory result	Cross-check	
	Positive	Negative
Positive	A	B
Negative	C	D

where:

A is the number of slides reported as positive by both readers (true positives);

B is the number of slides reported as positive in routine testing by the laboratory but found to be negative by the cross-checker (false positives);

C is the number of slides reported as negative in routine testing by the laboratory but found to be positive by the cross-checker (false negatives); and

D is the number of slides reported as negative by both readers (true negatives).

$$\text{Percentage agreement in parasite detection} = \frac{(A + D) \times 100\%}{A+B+C+D}$$

(ii) QC based on monitoring the accuracy of differentiation of *P. falciparum* and non-*P. falciparum* (in slides reported as positive by the routine laboratory):

Laboratory	Cross-checking	
	P. falciparum present	*P. falciparum* not present
P. falciparum present	A	B
P. falciparum not present	C	D

where:

A is the number of slides reported as containing *P. falciparum* (as a single or a mixed infection) by both readers;

B is the number of slides reported as containing *P. falciparum* only in routine testing by the laboratory but not confirmed by the cross-checker (incorrect species identification);

C is the number of slides reported by the laboratory as not containing *P. falciparum* in routine testing, but *P. falciparum* found to be present by the cross-checker, as a single or a mixed infection (incorrect species identification); and

D is the number of positive slides reported as not containing *P. falciparum* by both readers.

$$\text{Percentage agreement in species identification} = \frac{(A + D) \times 100\%}{A+B+C+D}$$

10.4.7 Statistical analysis

QC results should be analysed monthly and in a progressive 4-month cohort analysis. The analysis and reporting of the results of cross-checked slides should be standardized to avoid misunderstanding between validators and those whose performance is being checked.

To be meaningful, results must be robust, so that chance plays little or no part; they may have a considerable influence on staff morale and even on employment. Therefore, the threshold for incorrect results that trigger corrective action must be higher than that expected of a reasonable microscopist. This threshold requires benchmarking: determining what constitutes a reasonably achievable standard by comparing many results. Poorly performing laboratories can be identified in this way and given help by improving competence, deficits in materials or workplace conditions.

The system described above provides a representative sample for a slide cross-checking scheme. Experience with this method has shown that a mean of 97.4% of slides are interpreted correctly.

Monthly analysis of QC results

Individual monthly results should be evaluated for any major errors, to allow rapid feedback. Because of the small sample size, however, the result will not necessarily reflect the true overall performance of the laboratory:

- There may have been an exceptionally high workload, a problem with a reagent or a new staff member at the laboratory during the month, which should be reported centrally.
- Errors are not necessarily evenly distributed, and there may have been more errors than usual during a particular month; this may be balanced by a lower than normal error rate in another month.

- A limitation of a sample size of 10 is that single errors significantly affect the calculated percentage agreement. Hence, a single error in 10 QC samples will reduce the agreement to 90%.

Interpretation of individual monthly results should take into account the previous performance of a laboratory or test centre. The following may be used as a guideline.

When the previous QC results have been good to satisfactory

- Two errors out of 10 results is an alert.
- Three or more errors out of 10 results require immediate investigation.

When the previous QC results have been poor

- A result that is better than previous results is encouraging.
- A persistently static or a progressive decrease in the percentage agreement indicates that corrective action has not been effective and should be reviewed.

Progressive 4-month analysis of QC results

A progressive cohort analysis gives the percentage agreement, the percentage of false positives and the percentage of false negatives during the past 4 months. That is to say:

- After 4 months, use data for months 1, 2, 3 and 4.
- After 5 months, use data for months 2, 3, 4 and 5.
- After 6 months, use data for months 3, 4, 5 and 6.

Rationale

The disadvantages of analysis by month can be addressed by analysing QC data over a longer period, such as every 3, 4, 6 or 12 months. While a single error will reduce an individual monthly result to 90% agreement, it will reduce a 4-month result to only 97.5%. This allows more accurate assessment of laboratory performance

A balance must be achieved, however. Although greater accuracy is achieved by analysing a larger sample (for example, analysis of 6 months of data will be more accurate than that of 3 months of data), analysis of a smaller sample more efficiently detects recent changes in laboratory performance. There is little value in determining the performance of a test centre in the distant past, as circumstances will have changed significantly.

Calculation of 4-month indices

$$\text{4 month percentage agreement} = \frac{\text{Percentage agreement in months 1+2+3+4}}{4}$$

$$\text{4-month percentage false positives} = \frac{\text{Percentage false positives in months 1+2+3+4}}{4}$$

$$\text{4-month percentage false negatives} = \frac{\text{Percentage false negatives in months 1+2+3+4}}{4}$$

Interpretation

In **benchmark analysis**, QC benchmark standards are set annually on the basis of both the actual performance of field-testing, as determined by the QC protocol, and the overall goal for the accuracy of testing required for effective clinical diagnosis.

In quartile analysis, individual test centres assess their performance relative to that of other test centres; QC data are analysed centrally and reported as quartiles:

- 1st quartile: the highest result achieved by the 25% poorest performing test centres
- 2nd quartile: the median result achieved by all test centres
- 3rd quartile: the highest result achieved by 75% of test centres
- 4th quartile: the highest result achieved by one or more test centres

The advantage of progressive cohort analysis is that laboratories can analyse QC data with a statistically valid sample size (n=40) every month. A disadvantage of all cohort analysis is that performance above or below the average in any one month will distort the analysis for the total period.

Example: In a laboratory in which QC is performed on 10 samples per month:

Month	No. of errors	Monthly agreement	Progressive agreement
January	0	100%	Too few samples
February	1	90%	Too few samples
March	0	100%	Too few samples
April	1	90%	95%
May	3	70%	85.5%
June	0	100%	90%
July	1	90%	87.5%
August	0	100%	90%
September	1	90%	95%
October	0	100%	95%
November	1	90%	95%
December	0	100%	95%

Thus, a single poor result in May affects the progressive 4-month analysis for the period May to August. This disadvantage of cohort analysis applies irrespective of whether it is a progressive or a fixed-period analysis. With the above data, the same distortion occurs when the data are analysed in three fixed periods; for example:

- January–April: insufficient data
- May–August: 90%
- September–December: 95%

This distortion can apply in the opposite direction, when atypically good performance in one month results in overestimates of the actual laboratory performance for several months afterwards. This effect must be taken into account in analysing QC results.

Calculation of the true false-positive rate

The true false-positive rate is calculated on the assumption that there is little probability that strongly positive slides are false positives. If a blood film is reported by a laboratory as being strongly positive but found to be negative on cross-checking, this probably represents a clerical error rather than a technical reading error.

To calculate the true false-positive rate, laboratories and test centres must record the proportions of strong and weak positives reported in the period of the analysis:

True false-positive rate =

$$\frac{\text{Percentage of false-positives x total number of weakly positive blood films}}{\text{Total number of positive blood films}}$$

Example: Over 4 months, a laboratory reports 500 positive blood films, comprising 450 strongly positive and 50 weakly positive results. During the same period, 20 weakly positive thick films are randomly selected for cross-checking (five each month), and two are found to be negative.

The false-positive rate = 2/20 = 10%. As the total number of weak positives in this period is 50, by extrapolation, the estimated number of false-positive thick films = 5 (10% of 50). It is assumed that all strong positives are true positives (or clerical errors). The total number of positive slides in this period is 500. Therefore, the calculated true false-positive rate = 5/500 = 1%.

Benchmarking

Benchmarking allows individual laboratories and test centres to compare their performance with that of other laboratories and test centres. It also indicates realistic goals for sensitivity, specificity and accuracy on the basis of actual performance in field conditions. This can be achieved only by:

- central reporting and analysis of results and
- feedback of comparative data to the participating laboratories and test centres.

10.4.8 Reporting results

Monthly QC results should be reported to the QC supervisor within 2 weeks of the end of the calendar month in which routine testing was performed. Results should be reported on a standard QC reporting form. Model forms for reporting the results of cross-checking of slides by an external validator are given in Annexes 3 and 4.

10.5 Corrective action to be taken in the case of discordant results

One of the main benefits of effective QA is early recognition of problems and swift corrective action. Corrective actions must be taken whenever nonconformity is identified by cross-checking. If deficiencies in the cross-checking programme are identified, technical corrections and effective mechanisms to prevent recurrence must be introduced. This will ensure continuous quality improvement.

When cross-checking is performed by a reference laboratory or by a laboratory supervisor with greater competence than routine laboratory staff, the result should generally be considered correct. If the laboratory staff who performed the initial testing consider that the cross-checked result is incorrect, they should be given the opportunity to re-examine the slide or sample. Thus, microscopy slides sent to a reference laboratory for cross-checking and found to be discordant should, if possible, be returned to the routine laboratory after examination.

When cross-checking is performed by people with competence similar to that of the staff who performed the initial testing, any discrepancies should be reviewed by the original laboratory.

- If the laboratory that performed the initial reading agrees with the result of cross-checking (that the original reading was erroneous), the cross-check result can be accepted. This must be recorded as an error in the QC analysis.
- If the laboratory that performed the initial reading disagrees with the result of cross-checking, the slide or sample should either be re-examined by the cross-checker or referred to a third reader. If the cross-check result is found to be erroneous, the original result should be recorded as correct in the QC analysis.

10.6 Measuring the impact of cross-checking malaria slide results

Indicators that can be used to measure the impact of cross-checking of malaria slide results include:

- evidence of an effective laboratory cross-checking programme (such as schedules and results);
- up-to-date cross-checking records and feedback kept at the diagnostic facilities;
- improved accuracy and reliability of laboratory results over time; and
- evidence of an improving laboratory measurement system.

11. PROFICIENCY TESTING SCHEME

11.1 Terminology and definitions

Assigned value	True result or value of a test item; target or expected result
Advisory group	Panel of independent experts who mediate proficiency testing as needed
Challenge	A sample or test item to be assessed
Cycle	Number of surveys in a year (not restricted to a calendar year); also known as a “round”
Proficiency testing provider	Institute or laboratory coordinating the proficiency testing scheme; also known as the “proficiency testing coordinator”
Participant	Laboratory enrolled in the proficiency testing scheme
Referee	Laboratory (usually an alternative reference or tertiary level laboratory) selected to provide independent target values for test samples or verify the quality of test samples
Survey	A panel of challenges or samples

The term “external quality assessment” is used to describe a method by which an individual or body outside the laboratory, often the supervisor or governing authority, assesses a laboratory’s testing performance. This can be compared with the performance of a peer group of laboratories or a reference laboratory. The term is sometimes used interchangeably with “proficiency testing”; however, external quality assessment can also be conducted in other ways.

In proficiency testing, many samples with undisclosed results are regularly sent to a group of participating laboratories for analysis or identification. The results from each laboratory are compared with those of the other laboratories or with an assigned value, often set by a group of referee (validating) laboratories. Feedback on the performance of each participating laboratory is then sent to the laboratory and to other authorized entities, often a supervisor or governing authority.

In proficiency testing schemes, test laboratories examine a set of prepared slides received from an international, national or provincial reference laboratory, in order to gauge the ability of technicians to recognize, identify and count malaria parasites on the reference slides. Inter-laboratory comparisons are an important component of regular external quality assessment of a laboratory’s performance. Participation in a malaria proficiency testing scheme is compulsory for laboratories planning to upgrade their quality standard to achieve accreditation for malaria testing.

National reference laboratories should participate in proficiency testing schemes conducted by an established external provider, while intermediate and peripheral laboratories should participate in proficiency testing schemes conducted by a national provider.

11.2 Objective

Malaria proficiency testing schemes may be used to:

- assess the performance of a laboratory in providing accurate results;
- monitor a laboratory's continuing performance over time;
- identify problems or areas for improvement in malaria diagnosis;
- compare the performance of laboratories regionally, nationally or internationally;
- provide assurance to a laboratory's customers that it can provide accurate, reliable results; and
- provide training and educational materials to laboratories.

Ultimately, participation in proficiency testing is considered valuable if the information received from the scheme is used to improve the quality of malaria diagnosis.

11.3 Implementation

Effective QA should be conducted in a phased approach according to priorities, while proficiency testing is usually phased in as part of a fairly mature quality management system. Nevertheless, it could be started earlier if the basic requirements can be met. The activities listed below in priority order of introduction reflect, core activities (1-4); secondary activities (5-7); and final activities to implement a mature quality management system (8-11).

1. Baseline situation analysis: resources available in the country, but gaps in commodities and infrastructure
2. Identify the national core group of microscopists (undergoing ECA and certified at WHO level 1 or 2).
3. Establish a national steering committee.
4. Policies, guidelines, SOPs, with associated commodities and infrastructure
5. Competence assessment
6. Training
7. Supervision (outreach training and supportive supervision)
8. Cross-checking
9. Proficiency testing scheme
10. On-site evaluation
11. Diagnostic centre accredited to international standards such as ISO 9001:2008, ISO 15189:2012 or ISO 17025:2005

A malaria proficiency testing scheme should be designed to assess one or more of the diagnostic components of malaria microscopy:

Detection of malaria parasites in stained blood films: determination of whether a slide is positive or negative. Negative blood films should always be included.

Microscopic identification of malaria species in stained blood films: may be limited to the species prevalent in the area, or may include all species

Determination of malaria parasite density in stained blood films: may be assessed for accuracy and/or consistency. Accuracy can be calculated as the percentage deviation from the true count; acceptable counts are generally within 25% of the true count. Consistency can be assessed by sending duplicate slides and determining the percentage agreement between counts, which is usually acceptable if it is ≥ 75%.

Microscopy identification and identification of other blood pathogens in stained blood films: These may include microfilaria, trypanosomes, *Borrelia* and other pathogens.

Other techniques that can be tested: preparation of blood films, staining blood films, performing an RDT and paper- or web-based problems, in the form of photomicrographs. The last may be included but are not optimal. It should be noted that the first two, preparation and staining of blood films, may require participants to return slides to the proficiency testing provider for rechecking, which might be difficult logistically.

11.3.1 Implementers

Proficiency testing provider

In proficiency testing, laboratories receive samples from a provider, which may be an organization (non-profit or for-profit) formed specifically for proficiency testing, a central reference laboratory, a government health agency, an NGO or a manufacturer of kits or instruments.

The responsibilities of the provider are to organize and manage all the activities in a proficiency testing scheme, including:

- coordinating the enrolment of participating laboratories, including preparing and signing memoranda of understanding, as necessary, and handling fees;
- selecting, preparing and providing proficiency testing panels to the participating laboratories;
- preparing instructions for handling and processing samples for testing;
- receiving and analysing responses or answer sheets from the participating laboratories;
- communicating the results to participating laboratories (and to ministries of health and regulating bodies if necessary), including recommendations;
- providing educational materials and advice for improving poor performance;
- creating and administering a feedback or evaluation system to participating laboratories;
- maintaining a database of information on each discipline in each laboratory and performance over time;
- establishing formal agreements on the terms of reference of the advisory group and referee laboratories;
- annually evaluating the performance of the proficiency testing scheme and planning activities for the coming year; and
- identifying factors in participating laboratories that contribute to unsatisfactory performance, providing policy advice to ministries of health and providing follow-up or technical assistance as needed.

Advisory group

It is important that the scheme include individuals who are independent of the proficiency testing provider and have the necessary technical expertise to provide advice and mediate any conflict with participants. The responsibilities of the advisory group are to:

- provide technical advice to the provider, including modalities of implementation;
- assist in responding to participant queries or appeals when the provider and participant do not agree; and
- check documents before they are distributed by the provider to participants, as required.

Referee laboratory

A laboratory with known expertise, preferably with international accreditation (e.g. ISO 15189), tests the panels in the same way as the participants. Their results are often used to set target values and maintain an acceptable standard of the proficiency testing scheme. For example, if the referee laboratory does not obtain the expected result for a sample, the challenge might have to be assessed more leniently or not at all. The responsibility of the referee laboratory is to examine the samples in a routine way and return the results to the provider.

Participating laboratory

A laboratory that has enrolled in the proficiency testing scheme is responsible for:

- confirming its enrolment and providing correct contact information to facilitate prompt receipt of panels and other communications;
- paying fees promptly if a charge has been agreed with the provider;
- following the instructions of the provider;
- examining samples in the same way as routine samples;
- allocating duties to the staff who will process the panels;
- reporting any problem with the panels to the provider;
- returning results within the agreed time; and
- taking corrective action on all unacceptable results, which usually involves reviewing slides for unacceptable challenges, retraining or repairing or replacing equipment. If necessary, the laboratory should collaborate with the provider to address any problems found in proficiency testing.

11.3.2 Design of a malaria proficiency testing scheme

Basic resources

- a budget to cover at least a 2-year cycle;
- adequate staff with the competence required to coordinate the scheme;
- a laboratory with the necessary space and equipment to prepare the proficiency testing panels;
- a malaria slide bank to provide samples for the scheme;
- referee laboratories and/or an advisory group to ensure that an acceptable standard is maintained and/or to set target values;
- a reliable courier or postal service; and
- a database for registering participating laboratories, results and feedback.

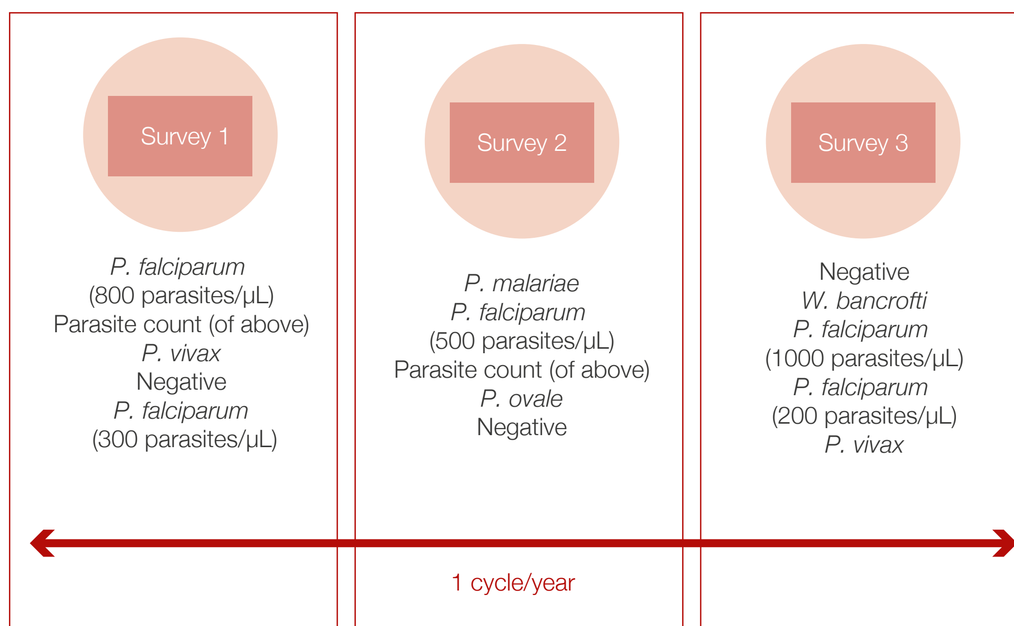
Frequency of survey testing and number of challenges per survey

- number of proficiency testing challenges and surveys per cycle appropriate to the volume and activity of the participating laboratories;
- at least three or four surveys per cycle. The most basic scheme should consist of at least five challenges per cycle; more may be added for assessment of the competence of reference or malaria research laboratories.

Selection and composition of surveys

- the species sent may be limited to those prevalent in the area or may cover all species. Negative blood films and films with low parasite counts should always be included. The composition of slides can vary from year to year or cycle.
- Fig. 6 gives *an example* of a cycle composition, with three surveys, each with five challenges.

Figure 6. Possible composition of an annual cycle



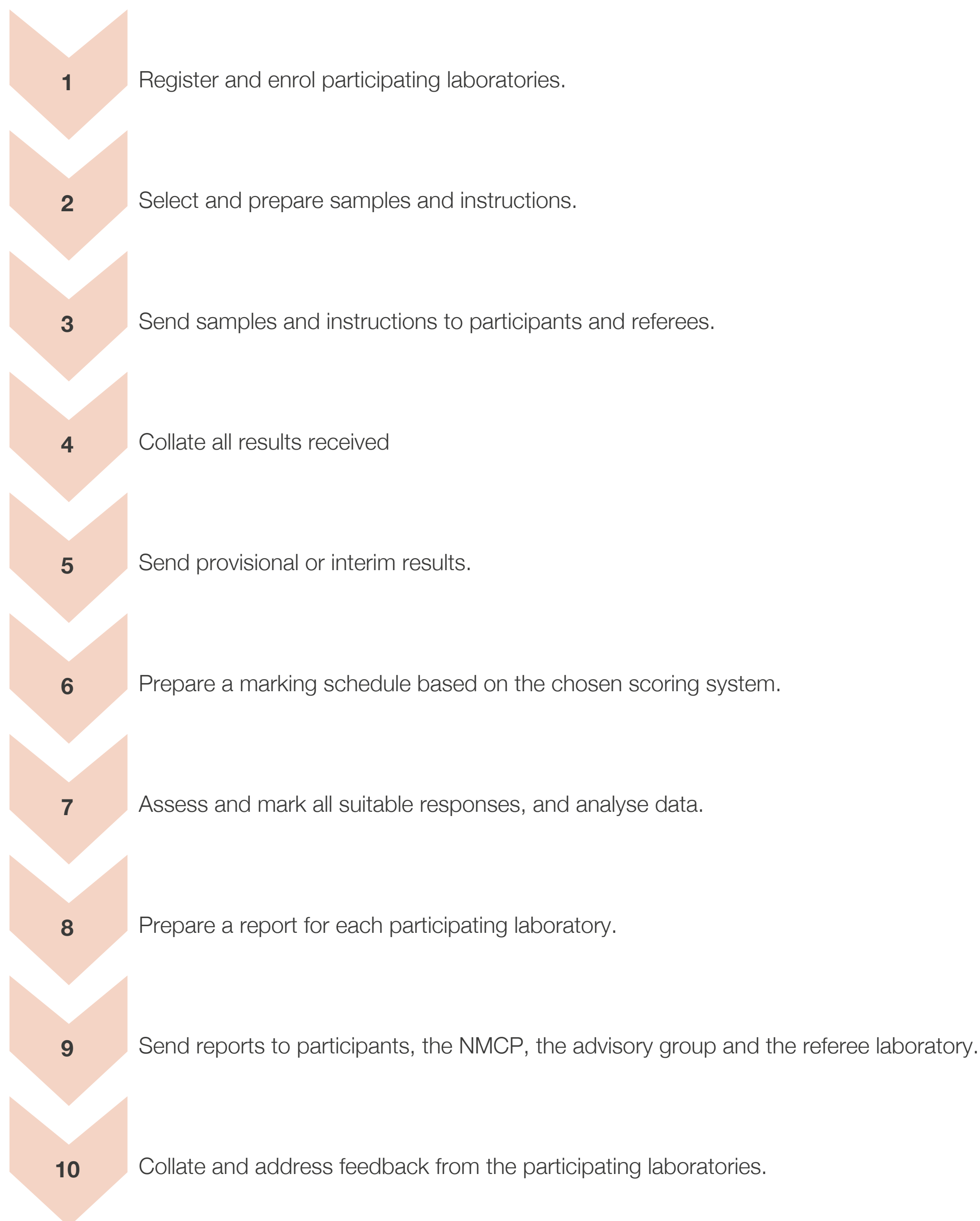
Data management and analysis

- There should be a procedure for determining the assigned value and a pre-prepared marking schedule.
- A validated data system for capturing and analysing results is required, which could be a Microsoft Excel or Access database or a web-based system.
- Tools are available in the public domain to improve the comparability of parasite detection in proficiency testing, such as http:archiverbm.rollbackmalaria.org/countryaction/docs/warn/LiberiaMicroReport.pdf.
- Standard reporting formats should be used. Reports may be automatically generated by the data system cited above.
- Annual feedback should be aggregated to determine the trend in the performance of a participating laboratory.

11.3.3 Process flow of a proficiency testing scheme for the provider

The basic process flow to be followed by the coordinator of a proficiency testing scheme is shown in Fig.7

Figure 7. Flow of activities of the proficiency testing provider



1. Register and enrol participating laboratories.

As most proficiency testing schemes are voluntary, it is important to obtain a written commitment from each participating laboratory to prevent waste of time, effort, samples and funds. Inform the participants about the procedures of the proficiency testing scheme and planned shipment dates for slides. The confidentiality of the results should be emphasized at this stage, and each participant should be assigned a code. In some instances, the provider may be required to forward laboratory results to the ministry of health, immediate supervisor, regulatory bodies or supporting partner.

If a fee is applicable, provide the banking information to participants before shipping. The fee must be agreed upon as part of the written commitment and received at the beginning of each annual cycle.

2. Select and prepare samples and instructions.

It is essential to ensure the homogeneity and stability of samples during their preparation. Samples should be prepared in the same way as routine patient samples, as far as possible. Therefore, all challenges should be thick and thin blood films. Participants should be instructed to treat proficiency testing samples in the same way as they would routine samples.

To avoid problems with illegible handwriting, answer codes may be used (Table 19).

Table 19. Example of answer codes for use in a proficiency testing scheme

Answer code	Microscopy answer
P1	No parasite seen; negative
P2	*P. falciparum*
P3	*P. malariae*
P4	*P. ovale*
P5	*P. vivax*
P6	Unable to identify the *Plasmodium* species
P7	Unable to identify whether *P. ovale* or *P. vivax*
P8	Other blood pathogen (with the name next to the code)

The deadline for results and submission details (e.g. fax number) must be clear.

3. Send samples and instructions to participants and referees.

If possible, a courier service should be used to deliver samples so that the package can be tracked; otherwise, participants should be asked to acknowledge receipt of samples.

Detailed instructions on how to analyse, answer the challenges and report back to the proficiency testing provider should be included in the package. A standard reporting form should be prepared (if a web-based system is not used).

The package may also include a letter from the proficiency testing provider (head of laboratory) that the slides/panels are safe, not hazardous or noncommunicable.

4. Collate all results received.

Laboratories should be allowed to decide how they will send their results, i.e. by post, fax, e-mail or on line. A reminder may be sent before the deadline.

5. Send provisional or interim reports.

This is optional but allows participants to take prompt corrective action if necessary.

6. Prepare a marking schedule based on the chosen scoring system.

The marking schedule may be based on the assigned value alone or on consensus between the referee and the participating laboratory. It can be organized before results are received. The marking schedule should clearly differentiate acceptable from unacceptable performance. Some schemes may apply negative marks to clinically dangerous answers.

A cut-off score could be set for results that pass the proficiency testing survey. An overall percentage of 80% is suggested.

An example of a possible scoring system is shown in Table 20.

Table 20. Possible scoring system for proficiency testing

Score	Result	Definition	Performance assessment
4	Completely correct result	Detection or identification: result accepted as the most correct and clinically relevant Count: within 25% of the true count	Acceptable
3	Almost completely correct result	Detection or identification: result not entirely correct but with little or no clinical impact; a deviation from what is considered the most clinically relevant result Count: 26–50% of the true count	Acceptable
Cut-off		*To divide acceptable from unacceptable responses*	
1	Significantly incorrect result	Detection or identification: a clinically relevant result that could lead to a minor error in diagnosis or treatment Count: 51–75% of the true count	Unacceptable
0	Completely incorrect result	Detection or identification: a clinically relevant result that could lead to a major error in diagnosis or treatment Count: > 75% of the true count	Unacceptable
0	No result, not returned	No result submitted by participating laboratory	Unacceptable

Another example of how results could be scored for panel testing is shown in Table 21.

Table 21. Scoring of panel slides for proficiency testing

Diagnostic criterion	Points per slide
Positive slide reported as *negative* or vice versa	0
Positive slide reported correctly as *positive*	3
Positive slide reported with correct parasite species identification	3
Positive slide reported with correct parasite stage identification	2
Positive slide reported with correct parasite load	2
Negative slide report correctly as *negative*	10

Each of the 10 panel slides is worth 10 points. For a parasite load count with the WBC method on a thick blood film, a variation ± 25% of the mean is acceptable.

An example of how results could be interpreted for panel testing is shown in Table 22.

Table 22. Interpretation of scoring of panel slide results

Score per slide	Definition	
	If correct for	If incorrect for
10	■ Parasite species identification ■ Parasite stage identification ■ Parasite load	
10	■ Negative slide reported correctly	
8	■ Parasite species identification ■ Stage identification	■ Parasite load
8	■ Parasite species identification ■ Parasite load	■ Parasite stage identification
6	■ Parasite species identification	■ Parasite stage identification ■ Parasite load
5	■ Parasite load	■ Parasite species identification ■ Stage identification
0		■ Positive reported as negative or vice versa

An example of how laboratory performance could be graded is given in Table 23.

Table 23. Grading of laboratory performance from the results of panel slides

Grade	Cumulative score (%)	Action
Excellent	≥ 90	Congratulate staff for exemplary performance.
Very good	80–< 90	Congratulate staff for very good performance, and tell them to maintain it.
Good	70–< 80	Congratulate staff for good performance, and tell them that further improvement is required. Check staff competence. Consider on-the-job training for identified weaknesses. Check reagent quality. Check microscopes.
Poor	≤ 70	Inform staff of the poor grading, and tell them that immediate action for improvement is required. Arrange immediate on-site supervision. Check staff competence. Consider on-the-job training for identified weaknesses. Check reagent quality. Check microscopes. Institute regular follow-up for corrective action.

7. Assess and mark all suitable responses, and analyse data.

Unsuitable responses may include returns received after the deadline or that are incomplete.

Analyse the data and use summary statistics, e.g. percentage of participants with acceptable responses or number of non-responding laboratories.

8. Prepare a report for each participating laboratory.

The reports should be concise and easy to interpret. They should detail the laboratory's performance and also provide an anonymous summary of the performance of all the participating laboratories in that survey or round. The report may also include the laboratory's performance in the previous one or two surveys to show trends. A corrective action form should be sent at the same time as the results, to encourage laboratories to take action on all unacceptable results.

Summary reports could also be prepared for the ministry of health, regulatory body, supporting partner and others.

9. Send reports to participants, the NMCP, the advisory group and the referee laboratory.

If a web-based system is used, participants can access their results online; however, the results must be available online only after the close of the survey. Teaching commentaries or materials could be included to address common errors and improve the overall performance of participating laboratories.

Certificates of participation or good performance could also be sent. These should be sent annually to cover one complete cycle.

10. Collate and address feedback from the participating laboratories.

To ensure that the needs of the participating laboratories are met and to improve the proficiency testing scheme, the provider should request regular feedback from participants. This can be done by sending a questionnaire, with questions such as "Is your laboratory satisfied with the coordination of this proficiency testing scheme?" and "What can we do to improve the proficiency testing scheme for you?". Otherwise, feedback could simply be requested on the coordination of the scheme.

Participants' feedback should be examined as soon as possible and responses given if necessary.

11.4 Corrective action

When the results of proficiency testing are received, laboratories must take corrective action rapidly for all unacceptable results or errors. This usually involves reviewing all the slides with unacceptable results, retraining or repairing or replacing equipment. If necessary, the provider can be asked to collaborate in rectifying the problems identified. If clinically dangerous results are noted, the provider may contact the laboratory or immediate supervisor directly.

As part of an effective quality management system, all external quality assessment activities must be formally recorded in a log book, including nonconformity and the corrective action taken, for example on a corrective action form. Details of nonconformity and corrective actions taken must be discussed with all staff and duly recorded. The results of proficiency testing must be discussed at regular laboratory meetings.

11.5 Measuring the impact of proficiency testing

The impact of a proficiency testing programme can be measured when a laboratory is assessed for accreditation, for example for compliance with ISO 15189. Other means could include:

- checking data on accuracy and precision recorded in the work log books of individual microscopists;
- assessing the results of internal audits;
- requesting regular feedback from participants, for example to determine whether they are more confident; and
- requesting regular feedback from clinicians.

12. REFERENCE MALARIA SLIDE BANKS

12.1 Background and objectives

Programmes to assess and improve the competence and performance of malaria microscopists have increased the demand for slide banks containing well-characterized, high-quality national reference malaria slide sets. The slide banks are used for continuous training and assessment of malaria microscopists in clinical settings and also of microscopists who may manage and supervise national QA programmes.

National programmes should set up their own slide banks to support their QA programmes, such as an NCA programme and national refresher training. Regional slide banks may be set up by international health organizations, research institutions or similar bodies. They should supply well-characterized malaria blood films for international ECA programmes, such as that outlined in section 6.

The objectives of slide banks are to provide:

- sets of known, replicate slides for training or assessment in malaria microscopy and QA;
- a permanent reference collection of the malaria species present in the country; and,
- on request, sets of reference slides from outside the country.

The achievement of these objectives will depend on the availability of adequate human and financial resources. If only limited resources are available, more limited targets should be set. All staff should have a clear understanding of the objectives and how they will be achieved.

12.2 Constitution of a slide bank

National or regional reference slide banks should contain, as a minimum, slides of all the malaria species currently found in the region or country, as well as blood slides that have been confirmed as malaria-negative. When an effective system is in operation and financial and human resources are available, consideration should also be given to the inclusion of slides of local zoonotic species that may affect individuals or communities, such as *P. knowlesi* in parts of South-East Asia, and other blood parasites common to the country, such as microfilaria, trypanosomes and *Borrelia* species. The number of slides in each category should reflect the prevalence of the parasite.

The size of the slide bank must be determined by:

- the number of ECA and NCA courses to be held each year,
- the state of development and characteristics of the QA system,
- other agencies that may be granted access and
- available resources.

A policy should be developed on access to the slide bank.

The species on all slides should be determined and confirmed in a quality-assured laboratory, when possible, by molecular methods such as PCR.

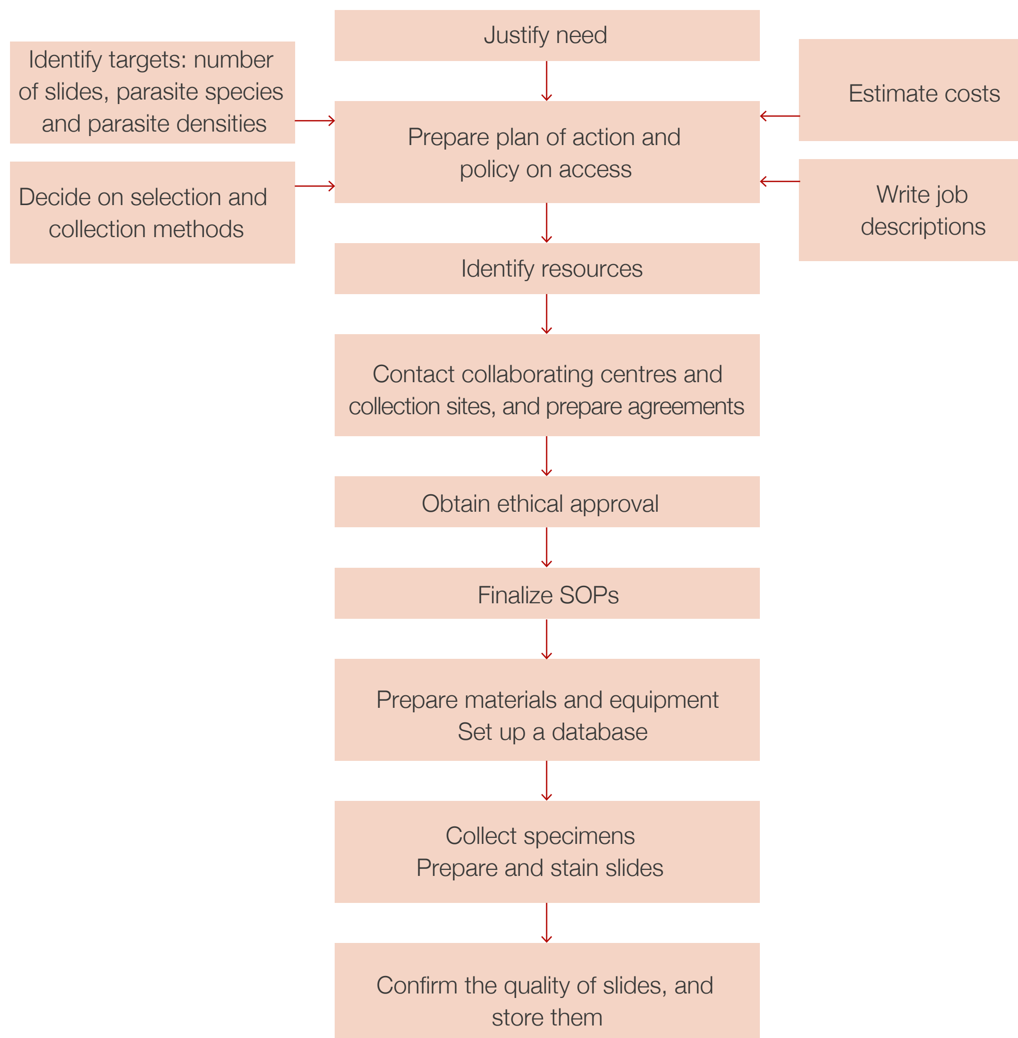
Experience shows that a slide bank is not sustainable without an effective plan of action, adequate funding and the commitment of competent laboratory and medical staff. Ethical clearance is required for the collection of samples, and there must be high-quality supplies and reagents, careful slide preparation, effective logistical and laboratory support and an efficient archiving system for practical retrieval of data and slides for dispatch and replenishment. Careful planning of slide bank activities is, therefore, critical.

The NRL should be responsible for establishing and maintaining a national (or in some instances regional) slide bank and preparation of a plan for its constitution and maintenance. The essential steps in a plan of action are:

- assessment of the requirements for establishment and maintenance of a slide bank;
- clear objectives and goals based on a “needs assessment”;
- a list of proposed activities, with a timetable for implementation, including:
  - selection of responsible staff,
  - determination of the composition of the bank,
  - selection of methods to collect slides,
  - determination of the ethical issues related to donor selection and
  - determination of the conditions of storage and supply of slides to users; and
- a realistic budget and assured availability of sufficient financial resources to sustain activities.

The specific objectives of a slide bank, its content, constitution and use should be determined by NRL staff in consultation with colleagues responsible for training and supervision in the QA programme of the NMCP and the ECA and NCA programmes. Fig. 8 illustrates the steps in establishing a slide bank.

Figure 8. Essential steps in establishing a slide bank



12.3 Costing

Establishment and operation of a slide bank facility require adequate, sustainable funding; if the project is planned and implemented carefully, only modest funding will be required. Initially, some additional funding may be required for equipment and supplies, but, provided operations are well coordinated with the activities of the NMCP and general laboratory services, recurrent costs can be kept at an acceptable level.

The largest financial outlay will probably be for:

- staff training and orientation;
- per diem and travel, if slides have be collected from the field; and
- equipment and supplies.

The essential equipment and supplies are:

- slide storage boxes or cabinets (metal or wood),
- labelling device and labels (consider bar codes and a bar-code reader),
- mounting media,
- slide trays,
- microscope slides with a frosted end,

- cover-slips of various sizes for mounting blood films,
- detergent,
- micropipettes,
- cloths for drying slides,
- disposable syringes (5-mL capacity) and needles,
- EDTA-treated collecting tubes (5-mL capacity),
- protective latex gloves (not powdered),
- stains and reagents such as buffer salts (or tablets),
- desiccators and active (indicator-coloured) silica gel,
- record forms (coded) and registers,
- diamond-tipped objective marker (optional) and
- a digital camera and attachment for the microscope (optional).

12.4 Selection of staff

At least one senior laboratory technologist (the title may differ from country to country), ideally working within the NRL, should be responsible for the organization, planning and implementation of slide bank activities. This technologist should be highly skilled in all aspects of malaria parasite diagnostics and have proven ability to organize and oversee a small team of personnel trained in slide bank operations.

The senior technologist should be assisted by at least two junior staff, each working full time during establishment of the bank. Additional staff may be required for field or collecting activities, but these can be seconded and participate as needed, after prior agreement between units or departments.

The job descriptions of some or all of the above staff might have to be modified, and additional training might be required before they undertake slide bank activities. The change in job description and the additional training might be minimal, but both are essential so that all staff are fully aware of their additional responsibilities and can perform them effectively. Dedicated staff with specific training is essential.

12.5 Methods of slide collection

Slides can be collected at hospitals, at health centres or during field surveys. Staff should be aware that each method has its advantages and disadvantages and should carefully consider them; however, a final decision on which is the most appropriate must be based on local knowledge.

12.5.1 Hospital or health centre

Donors are selected from among referred patients or patients reporting to the outpatient department. Samples can be obtained in one of three ways.

- **Resident staff select the donor during daily routine and inform slide bank staff, who travel to the facility and take and process the blood sample.** If the health facility is readily accessible, this system works well.
- **Resident staff participate in routine activities, select a suitable donor, collect a sample and forward it rapidly to the slide bank for processing.** Timely dispatch of blood specimens and receipt by slide bank staff is critical, as

parasite morphology in EDTA-treated blood starts diverging too far from normal within about 1 h at room temperature. If rapid transport can be assured, this is a successful approach.

- **Slide bank staff based temporarily at the hospital select a suitable donor, collect a sample and process it.** This is an efficient approach but requires a steady supply of donors to be cost–effective. Space may be restricted in small health centres, and equipment and supplies must be transported to the facility. The centre should be informed that the visiting staff are collecting samples for a national slide bank set. The slide bank team can help resident staff in screening patients and referring any suitable cases to the team for blood collection before treatment is given.

12.5.2 Selective surveys

NMCP surveys or combined health activities in small communities are a good opportunity for collecting slide bank material; the chance of finding donors is high, and the method is cost–effective.

Previous experience will indicate the best time and areas. Species and parasite densities are usually seasonal. In hyper- and holo-endemic areas, cases occur all year, but the density is higher in the high-transmission season, when the incidence of all species increases, including *P. malariae* (and *P. ovale* when present). Communities with a high prevalence of malaria are often far from services and may be difficult to access. Combining slide bank activities with other health activities that benefit poorly served communities is often well accepted and effective. Establishment of an operations centre allows coverage of a larger area and population; therefore, working and living facilities must be considered.

Such regular activities ensure the provision of enough material for the slide bank and allow regular replenishment and updating.

12.6 Selection of donors

The selection of donors is the most important step in establishing a slide bank. It should be based on criteria set by the NMCP in accordance with the highest ethical standards. The infections likely to be seen by microscopists in the country must be known before selection begins. In malaria-endemic countries, parasite negativity might have to be verified by PCR or by using specimens from non-endemic countries.

The following should be established before samples are taken:

- the sampling method and case selection criteria,
- the minimum age of donors,
- that the donor is healthy,
- the parasite stages and species required,
- the range of parasite densities and
- whether unusual parasite forms and other blood parasites will be included.

The exclusion criteria should include:

- unwillingness to donate blood or give informed consent,
- any contraindication to donation of 3 mL of blood (at the discretion of the licensed project physician),
- recent (4 previous weeks) treatment with antimalarial drugs,
- severe or complicated malaria requiring emergency transfer for management and
- a history of bleeding.

Collection of samples for the slide bank should be based on ethical principles:

- all blood collection should be approved by the national institutional review board designated to oversee research and specimen collection involving human subjects.
- donors should not be paid for providing medical specimens.
- informed consent must be obtained after the donor has been told about the use of the specimen.
- the health and welfare of the donor is paramount and should not be compromised by the activities of the slide bank programme.
- provision should be made to treat malaria and other diseases likely to be encountered.

Each potential donor or their carer should be informed about the objectives of the programme and the reasons and procedures for taking a blood sample. Prospective donors, their carers or a responsible person must sign a consent form confirming that they understand the request and agree to collaborate in the programme.

The risks and discomfort of blood donation are minimal. The site may be painful for a few days after blood collection. Potential complications of venepuncture include fainting, bleeding, bruising, haematoma formation or, very rarely, local infection. All precautions should be taken to minimize the risk for infection. The risk for significant bleeding from venepuncture is minimal, but prolonged compression may sometimes be required.

Documents containing personal information on donors should be securely stored. No personal identifying information should be stored with the blood films once they are in the bank and accessible to users, and the samples should be accessible only to relevant staff. All information accompanying banked slides should be completely de-linked from data identifying the individual by use of a standard coding system. Access to and use of the bank should be limited to the terms covered in the consent obtained from the patient and the terms of approval of the relevant institutional review board.

12.7 Slide preparation and labelling

12.7.1 Slide preparation

WHO is formulating detailed SOPs for preparing large numbers of uniform, high-quality blood films, but these were not available at the time of publication of this *Manual*. It is recommended that SOPs from authoritative institutions be followed closely to ensure that batches of slides prepared at different times and at different sites are interchangeable. It is important to ensure that trainees and candidates being assessed are unaware of the site or date of collection.

Detailed planning is essential. The lists of equipment provided must be adapted to local requirements, and all the necessary material must be available before collection starts. The shorter the delay in producing films after venepuncture, the higher the expected quality of the films. A clear routine must be established, from the time of venepuncture to labelling, to prevent mixing up samples or films from different donors; batches should

be maintained separately at all times. Slide preparation should be like a production line, each technician having clear allocated tasks. Blood from a single donor should be processed (film preparation and staining) at one time to avoid cross-contamination or mistakes in labelling.

Multiple templates should be made for thick films, and cleaned slides should be laid out on a workbench in rows to allow rapid, accurate pipetting. Thick films should be spread rapidly and carefully onto the templates, ensuring consistent thickness across the film.

Once slides have been stained and dried, a selection must be labelled and put aside for validation. The remainder of the slides from each donor should be placed in sealed slide boxes and clearly labelled with the case code once validation is complete or on site. A bar code would blind future readers to the details of the case (see below).

12.7.2 Dilution to selected parasite densities

When certain parasite densities are not available in the community being sampled, films of the selected parasite density can be produced by dilution with parasite-negative donor blood. This should be avoided if possible, as complications such as clumping of cells may occur. A protocol for diluting blood can be adapted from SOP 3.2 of the WHO *Methods manual for laboratory quality control testing of malaria rapid diagnostic tests* (version 5a), 2008 (www.wpro.who.int/sites/rdt).

12.7.3 Labelling

Slides should be labelled on the bevelled area clear of the blood film and cover-slip. For clarity, printed labels should be used, on high-quality labelling paper that will adhere after years of use. Use of a bar code label printer and bar code reader should be considered, to allow blinding of readers who are undergoing competence assessment. Removal and return of slides to the bank could be recorded in the database with the bar code reader, making future operation of the bank and tracking of slides more efficient and accurate.

12.8 Data management and entry

Data management should be based on the use of standard reporting forms. Names and other unique information on donors should not be kept in permanent records. A temporary log linking donors' numbers to their names is useful during sample collection but must be destroyed at the end of each day. The data to be recorded is listed below.

Demographic data

- number
- age
- gender
- locality
- history of malaria treatment
- history of travel (optional)

Malaria microscopy results

- positive or negative
- species present (*P. falciparum, P. malariae, P. ovale, P. vivax, P. knowlesi*)
- presence of gametocytes
- single or mixed infection
- parasite count per 500 WBCs and per 5000 RBCs
- parasite density per microlitre (determined from true WBC count)
- concordance or discordance with validation results (optional)

A computer database should be set up on the following principles:

- Only authorized users have access to the database, using a user name and password.
- Entries are made from the original reporting forms directly into the database.
- Double data entry and cross-validation are used, and discrepant results are checked against the original data.
- Records are backed-up regularly.

12.9 Slide bank storage and maintenance

The operation and maintenance of a slide bank is fairly straightforward, requiring little more than modest laboratory space for storage of slide sets. It must be clearly understood that the slide bank provides a service for various activities of the NMCP, with materials available on demand for training and external quality assessment. Sufficient stocks of slides should be maintained to meet the annual estimated requirements (from a needs assessment), so that there are no stock-outs or delays in dispatch. This requires an effective archiving and reporting system to track dispatched slides, current stock levels and their shelf life over time.

ANNEX 1. MODEL LIST OF EQUIPMENT AND SUPPLIES FOR A MALARIA DIAGNOSTIC LABORATORY

Equipment		
Item	**Quantity**	**Comments**
Microscope, binocular, with electric light source, x10 eyepiece, objective lenses (x 10, x 40, x 100 oil immersion)	1 per microscopist	
Microscope, multi-head (e.g. five), binocular, with electric light source, x10 eyepiece, objective lenses (x10, x40, x100 oil immersion)	1	Optional
pH meter	1	
pH (litmus) paper	1	If no pH meter
Pipette, adjustable volume, 5–20 µL	1	
Pipette, adjustable volume, 100 µL	1	
Pipette, adjustable volume, 100–1000 µL	1	
Tally counter, 2-key, 4 digits, hand-operated	1 per microscopist	
Microscope light, battery-powered, e.g. with white LED light	1 per microscope	If no reliable external power source
Calculator, battery-powered	1 per microscopist	
Heating tray or warming block, electric, to 37 °C	1	Optional
Timer, digital, 60 min with alarm	3	

Non-consumables		
Item	Quantity	Comments
Rack for slides, expandable, stainless steel	1	
Bottle, swan neck jet, plastic, 250 mL	2	
Funnel, plastic, 90 mm diameter at short end	2	
Rack for drying slides, vertical, plastic, holds 10 slides	3	
Cylinder, measuring, plastic, graduated, spout, 100 mL	1	
Cylinder, measuring, plastic, graduated, spout, 250 mL	1	
Funnel, 100 mm diameter, short end	1	
Spatula, double, for analysis, stainless steel	1	
Bottle, glass, brown, screw cap, 1 L	3	
Microscope light bulb	2 per microscope	e.g. 6 V, 20 W
Slide box, for 100 slides	12	
Beaker, graduated, glass, 100 mL	1	
Beaker, graduated, plastic, 100 mL	1	
Beaker, glass, Pyrex, 500 mL	1	

Non-consumables		
Item	Quantity	Comments
Staining jar, Coplin, glass, with lid	2	
Glass or polyethylene staining trough with lid (e.g. for 20 double-thickness slides)	5	
Spreader slides (for making blood films)	2	Optional
Diamond-tipped pencils	4	For labelling slides
Laboratory gowns (preferably long-sleeved, wrist cuff, no pockets, tied at rear)	1 per microscopist	Spares for visitors
Safety glasses	1 per microscopist	Spares for visitors

Consumables			
Item	Quantity	Typical packaging	Comments
Microscope maintenance and cleaning kit	3		Including lens tissue, lens cleaning solution, anti-static micro-fibre cleaning cloth, blower and anti-static brush Optional
Gloves, examination, latex, disposable, large	15 boxes	50 pairs/box	About 3 months' supply per person
Gloves, examination, latex, disposable, medium	15 boxes	50 pairs/box	About 3 months' supply per person
Gloves, examination, latex, disposable, small	6 boxes	50 pairs/box	About 3 months' supply per person
Lens cleaning solution	1	1 L bottle	
Marker, black, dye-, bleach- and water-resistant	6	Roll	
Cotton-wool, hydrophilic	1 roll (500 g)	500 swabs	About 3 months' supply
Lancet, disposable, sterile, standard type	10 boxes	200 lancets/box	About 3 months' supply
Sharps container, needles and syringes, 15 L, cardboard for incineration	10	Individual packaging	About 3 months' supply
Sharps container, needles and syringes, 500 mL, plastic	10	Individual packaging	About 3 months' supply
Biohazard waste box	6	Individual packaging	About 3 months' supply
Needle, sterile, 21 gauge	1 box	100 needles/box	About 3 months' supply
Tube, vacuum, EDTA, 3 mL	1 box	100 tubes/box	About 3 months' supply
Tube, vacuum, plain, 3 mL	1 box	100 tubes/box	About 3 months' supply
Tube holder, vacuum, plastic	2 boxes	100/box	

Consumables			
Item	Quantity	Typical packaging	Comments
Needle, vacuum tube, 21 gauge x 1.5”	6	100/box	
Syringe, disposable, 5 mL	1	100 syringes	About 3 months’ supply
Sampling set, with wings, 23 gauge	1 pack	200 /pack	About 6 months’ supply
Glass slide, 76 mm x 26 mm, 1.0–1.2 mm thick	60 boxes	50 slides/ box	
Filter paper suitable for PCR sampling	2 packs	100 discs/ pack	
Lens cleaning paper, sheet	1	100 sheets/ booklet	
Pipette, transfer (Pasteur), graduated, plastic, non-sterile	500		
Detergent	5 L		Local purchase
Chlorine, 1 g (sodium dichloroisocyanurate 1.67 g) tablets	100	100 tablets	1 tablet provides 0.2 L of a 0.5% chlorine solution. 100 tablets provide 10 L of a 1% solution
Pencil, grease, red, glass-writing	2		
Slide label, round, white and coloured, 14 mm diameter	1	Pack of 100	
Coverslip, glass, 24 mm x 50 mm	4	Pack of 100	
Mounting medium	3	Bottles of 100 mL	e.g. Depex
Alcohol swabs	6	Pack of 100	
Wound-covering strips	6	Pack of 100	
Tourniquet	3		
Reagents			
Iodine povidone, 10% solution	3	200 mL dropper bottle	About 3 months’ supply
Oil, immersion	1	500 mL bottle	Enough for 50 µL of oil (1 drop) for about 10 000 slides
Isopropyl alcohol	1	1 L bottle	
Methanol	2	1 L bottle	About 2000 slides can be fixed with 1 L
pH calibration solution, pH 4.0	1	250 mL	
pH calibration solution, pH 7.0	1	250 mL	
pH calibration solution, pH 10.0	1	250 mL	
Cleaning solution (pH meter), general purpose	1		

Consumables			
Item	Quantity	Typical packaging	Comments
Potassium dihydrophosphate	1	500 g	
Disodium hydrogen phosphate	1	500 g	
Buffer, pH 7.2	100 tablets		One tablet for 1 L
Giemsa stain	10	500 mL bottles	

Manuals and documents
NMCP standard operating procedures for malaria microscopy
Bench aids for the diagnosis of malaria infections. 3rd Edition. WHO, 2009.
Basic malaria microscopy, Part 1, Tutor's guide. 2nd Edition. WHO, 2010.
Basic malaria microscopy, Part 2, Learner's guide. 2nd Edition. WHO, 2010.
Malaria microscopy quality assurance manual. 2nd Edition WHO, 2015.

ANNEX 2. EXAMPLES OF CHECKLISTS AND REPORTING FORMS FOR SUPERVISORY VISITS

The supervisor should ensure that all the following are checked.

Organizational issues

- Written SOPs are available.
- An adequate supply of reagents within the expiration dates is available.
- Well-maintained microscopes of high quality are available.
- There are satisfactory supplies of good-quality reagents and consumables.
- Internal QC is performed at the required intervals.
- Laboratory safety practices are observed.
- Record-keeping is accurate and consistent with the requirements.
- Results are reported promptly to the treatment centre or clinicians.
- Patient slides are available and properly stored for cross-checking during consultative visits or after submission to the intermediate level.
- Staff have received adequate training, with refresher courses or corrective action recommended when appropriate.
- The workload is monitored and is satisfactory.

Technical issues

- Blood slides are properly collected.
- Procedures for film preparation, staining and examination are in accordance with NMCP SOPs.
- Control slides known to be positive and negative are used to control the quality of each daily batch of buffer and stain before blood films from new patients are processed.
- Corrective action is taken to remedy errors and problems.
- Significant problems requiring strategies and systems for improvement are documented.

Supervisory visit report form for assessment of malaria microscopy laboratories

1. General information

Name of laboratory or facility				Date of visit (dd/mm/yyyy)
Type of facility:	☐ Rural clinic	☐ District hospital	☐ Provincial hospital	
	☐ Private hospital	☐ Private Laboratory	☐ Other (specify)_______	
Physical address of laboratory:				
Municipality, city, town:		Province:		
Telephone:	Fax:	E-mail		
Name of head of department or director of laboratory:				
Name of head of facility:				
Names of microscopists interviewed:				
Names of interviewers:				

2. Organization and management

	Yes	No	Remarks
Is the laboratory licensed to practise (for private laboratory)?			
Are the microscopists registered and licensed to practise (for private laboratory)?			
Is there a regular training programme for microscopists?			
Names of trained microscopists			
1 ____ Date of last training: ______- -______ No. of months in the laboratory: ☐ ☐ dd mm yyyy			
2 ____ Date of last training: ______- -______ No. of months in the laboratory: ☐ ☐ dd mm yyyy			
3 ____ Date of last training: ______- -______ No. of months in the laboratory: ☐ ☐ dd mm yyyy			
4 ____ Date of last training: ______- -______ No. of months in the laboratory: ☐ ☐ dd mm yyyy			
5 ____ Date of last training: ______- -______ No. of months in the laboratory: ☐ ☐ dd mm yyyy			

3. Documentation

	Yes	No	Remarks
Are pathology request forms available?			
Are results recorded in an organized and legible manner in log books?			
Are approved SOPs available in the laboratory?			
Are technical manuals and bench aids available in the laboratory?			
Are internal QC log sheets available?			
Are maintenance log books for microscopes and pH meters available?			

4. Procedures

Blood film preparation			
	Yes	No	Remarks
Are SOPs available for blood film preparation?			
Are both thick and thin films prepared?			
Are blood films labelled appropriately?			
Is the quality of prepared blood films monitored?			
Are unstained slides protected from insects and auto-fixation?			

Blood film staining			
	Yes	No	Remarks
Are SOPs available for blood film staining?			
Are recommended reagent preparation procedures followed?			
Is internal QC performed regularly with known positive and negative slides during staining?			
What is the staining technique used? ☐☐ Giemsa stain ☐ Other (specify)___________________________________			
For Giemsa technique:			
Is buffered distilled water pH 7.2 ± 0.2 used to dilute the Giemsa stain?			
Is the Giemsa working stain solution freshly prepared before each staining (within 4 h)?			

Blood film examination			
	Yes	No	Remarks
Are SOPs available for examination of blood films?			
Do microscopists routinely report the presence or absence of parasites, species and density?			
Do microscopists report parasite density in the recommended way (parasites/µL)?			WHO recommended method
Does the workload allow the recommended reading time (at least 10 min) per slide?			
Are examined slides stored and archived properly?			
Average number of slides read per month	☐☐☐		
No. of slides read per day (in highly endemic areas)	☐☐☐		
Average no. of slides read per day per microscopist (in highly endemic areas)	☐☐☐/microscopist		
No. of slides archived or assessed by validator	☐☐☐		

5. Competence assessment

Performance (review of slides), cross-checking	
No. of slides cross-checked by validator	☐☐
Parasite detection agreement (%)	☐☐
False positives (%)	☐☐
False negatives (%)	☐☐
No. of true positives	☐☐
Species identification agreement (%)	☐☐
Parasite density agreement (%)	☐☐
Poorly prepared thick films (consider size, shape and volume of blood) (%)	☐☐
Poorly prepared thin films (consider size, shape and volume of blood) (%)	☐☐
Slides poorly stained (%)	☐☐
Slides containing stain precipitate or artefacts (%)	☐☐
Slides auto-fixed (%)	☐☐

Reference slides provided by the supervisor and examined by the laboratory	Microscopist				
	1	2	3	4	5
No. of slides read	☐☐	☐☐	☐☐	☐☐	☐☐
Parasite detection agreement (%)	☐☐	☐☐	☐☐	☐☐	☐☐
False positives (%)	☐☐	☐☐	☐☐	☐☐	☐☐
False negatives (%)	☐☐	☐☐	☐☐	☐☐	☐☐
Species identification agreement (%)	☐☐	☐☐	☐☐	☐☐	☐☐
Parasite density agreement (%)	☐☐	☐☐	☐☐	☐☐	☐☐

6. Quality assurance

	Yes	No	Remarks
Does the laboratory comply fully with the national QA and QC guidelines?			
Is there a formal protocol for analysing internal QC results and taking corrective action if the results are not satisfactory?			
Do the laboratory or microscopists regularly participate in a proficiency testing scheme or other form of external QA?			
Is the performance of the laboratory or microscopists in the proficiency testing scheme or other form of external quality assessment satisfactory?			
Does the laboratory have procedures to address poor performance in proficiency testing or other forms of external quality assessment?			

7. Laboratory set-up and environment

	Good	Poor	Remarks
Bench space			
Sink, washing area, staining area			
Access to clean water supply			
Natural lighting			
Power source			
Ventilation			
Storage space for supplies and materials			
Storage space for unstained and examined slides			
Secure storage space for confidentiality of patient results			

8. Biosafety

	Yes	No	Remarks
Laboratory staff wear protective laboratory coats and gowns and safety glasses			No pockets, with tight cuffs
Staff wear gloves when collecting and handling blood samples			
Hand-washing facilities with soap (or similar) available			
Power supply for the microscope(s) and laboratory lighting in good condition and safe			
Proper disposal units			
Containers for dry waste			
Containers for infectious materials			
Puncture-resistant container for sharps and blood slides			
Disposal of waste materials as per national guidelines			

9. Equipment and reagents

Microscopes			
	Yes	No	Remarks
The microscope(s) is binocular with oil immersion x100 objective			
The microscope lamp(s) has sufficient power to provide good illumination when the condenser aperture is set at the correct setting for the x100 objective			
Blood films can be brought into sharp focus at x100 oil immersion magnification.			
The stage movement mechanism is precise and stable.			
The microscope is placed on a stable bench, with adequate working space and away from staining areas and vibration producing equipment such as centrifuges			
The microscope(s) is regularly serviced.			
The microscope(s) is cleaned and protected with a cover after use.			
Xylene is used to clean the microscope(s), objectives or eye pieces			Xylene is carcinogenic and should not be used.
Spare microscope bulbs are available.			

Microscope slides			
	Yes	No	Remarks
Microscope slides are of good quality and are thoroughly cleaned before use.			
Microscope slides do not have scratches or surface aberrations.			
Microscope slides do not give a blue background colour after staining.			
Microscope slides do not have fungal contamination.			
Microscope slides that have been damaged by fungus are discarded and not used again.			
In areas with high humidity, microscope slides are protected against fungal contamination.			
Microscope slides are re-used.			

Staining reagents			
	Yes	No	Remarks
All required staining reagents are available.			
All staining reagents are within the recommended expiry date.			
Staining solutions are stored as per the manufacturer's recommendations.			
SOPs are available for preparation of working stain solutions.			
Internal QC is performed for each batch of working stain solution prepared and each batch of commercially prepared stain opened for use.			
Commercial stain solutions do not contain excessive stain precipitate.			
The cap of the reagent bottle is always tightly sealed except when stain is being removed for use.			
Stain is always removed from the reagent bottle with a clean pipette or similar.			
Water is never added to the stock stain solution.			
Unused stain is never returned to the stock bottle.			

General laboratory supplies			
	Yes	No	Remarks
Alcohol and cotton (or similar) for cleaning skin prior to blood collection			
Lancets			
Methanol			
Giemsa stain			
Buffer salts or buffer tablets			
pH meter accurate to two decimal places			
pH calibration solutions			
Staining jar			
Microscope light bulbs			
Spreader (for making blood films)			
Laboratory gowns			
Safety glasses			including over-spectacles type
Gloves, disposable			
Lens cleaning solution			
Marker pens			
Sharps containers			
Needles and syringes			
Vacuum venepuncture supplies			
Pencils, grease, red, glass-writing			
Slide labels			
Coverslips			
Mounting medium			
Tourniquet			
Wound cover strips			
Staining rack			
Drying rack			
Graduated cylinders of the correct size			
Wash bottles			
Timers, sufficient number for staining for each microscopist			
Immersion oil of acceptable viscosity (not too thick and not too thin)			
Tally counters, sufficient number for the number of staff			
Lens paper			
Slide boxes for storage			
For laboratories in which stain is prepared from powder: sufficient glycerol, methanol, powder, beakers, measuring cylinders, filter paper, funnels, stirring rods, scales, spatulas and storage bottles			

10. Performance indicators

Monitoring of:	Yes	No	Remarks
Total number of slides examined			
Total number of positive slides, stratified by species			
Consumption of commodities			
Monthly stock-outs of microscopy reagents			
Turnaround time for microscopy results			

General findings and recommendations:

Supervisor or auditor's comments:

Accomplished by:

Name of auditor

Signature of auditor

Date: --

ANNEX 3. MODEL MONTHLY REPORTING FORM FOR CROSS-CHECKING MALARIA BLOOD SLIDES: NO SPECIES IDENTIFICATION

Cross-checking centre:

Laboratory name:

Month	A	B
	C	D
January		
February		
March		
April		
May		
June		
July		
August		
September		
October		
November		
December		

	Observer 2 - cross-checker		
Observer 1-laboratory	Positive	Negative	Total
Positive	A	B	A+B
Negative	C	D	C+D
Total	A+C	B+D	A+B+C+D

A = number of slides reported as positive by both readers

B = number of slides reported as positive in routine testing by the laboratory but found to be negative by the cross-checker (false-positives)

C = number of slides reported as negative in routine testing by the laboratory but found to be positive by the cross-checker (false-negatives)

D = number of slides reported as negative by both readers

NB: Enter the values for A, B, C, D in the 2 x 2 table for each month and calculate:

1. Percentage parasite detection (slide reading agreement) = (A+D) x 100% / (A+B+C+D)
2. Percentage false positive rate = B x 100% / (A+B)
3. Percentage false negatives = C x 100% / (C+D)

Send the completed reports to the QC supervisor

Comments on quality of blood film and staining:

Grade	Number of slides
Excellent	
Good	
Poor	

Criteria for assessing the quality of blood films

Grade	Criteria for assessing the quality of malaria blood film preparation and staining characteristics
Excellent	**Gross appearance:** Both thin and thick film prepared on the same slide, thick film 10 mm in diameter, newsprint could be read under thick film before staining, 10 mm from frosted end and thick film and between a thick and a thin film, with distinct head, body and tail
	Microscopy appearance: RBCs lysed in thick film and a monolayer of RBCs, with normal and abnormal morphology in thin film. Staining allows trophozoites, gametocytes and/or schizonts and WBC to be clearly distinguished against the background.
Good	**Gross appearance:** Film with uneven tail, too thick, too wide or too long, of uneven thickness
	Microscopy appearance: A monolayer of RBCs and fixed RBCs. Staining allows trophozoites, gametocytes and/or schizonts and WBC to be clearly distinguished against the background.
Poor	**Gross appearance:** Film with ragged tail, too thick, too wide or too long, of uneven thickness
	Microscopy appearance: Distorted appearance of RBCs, malaria parasites and WBC. Difficult to spot fields with monolayer of cells; distorted appearance of the RBCs, malaria parasites and WBC

ANNEX 4. MODEL MONTHLY REPORTING FORM FOR CROSS-CHECKING MALARIA BLOOD SLIDES: SPECIES IDENTIFICATION

Cross-checking centre:

Laboratory name:

Month	A	B
	C	D
January		
February		
March		
April		
May		
June		
July		
August		
September		
October		
November		
December		

	Observer 2 - cross-checker		
Observer 1- laboratory	***P. falciparum* present**	***P. falciparum* not present**	**Total**
P. falciparum present	A	B	A+B
P. falciparum not present	C	D	C+D
Total	A+C	B+D	A+B+C+D

A A = number of slides reported as containing *P. falciparum* (as either a single or a mixed infection) by both readers

B = number of slides reported as containing *P. falciparum* only in routine testing in the laboratory but not confirmed by the cross-checker (incorrect species identification)

C = number of slides reported as not containing *P. falciparum* in routine testing in the laboratory but found to be present by the cross-

checker as either a single or a mixed infection (incorrect species identification)

D = number of slides reported as not containing *P. falciparum* by

both readers

NB: Enter the values for A, B, C, D in the 2 x 2 table for each month

Percentage agreement on species identification = (A + D) x 100% / (A + B + C + D)

Grading the performance of parasite detection and species identification in slide rechecking

Grade	Percentage slide agreement		Action
	Parasite detection	Species identification	
Excellent	≥ 95%	≥ 85%	■ Congratulate staff on exemplary performance.
Very good	85≤ 95%	75≤ 85%	■ Congratulate staff on very good performance, and tell them to maintain it. ■ Identify any aspect for improvement.
Good	75≤ 85%	65≤ 75%	■ Congratulate staff good performance on, and tell them there is room for further improvement. ■ Conduct regular on-site supervision. ■ Check staff competence. ■ Check reagent quality and the microscope. ■ Consider on-the-job training to remedy weaknesses.
Poor	≤ 75%	≤ 65%	■ Inform staff about poor performance, and tell them that immediate action is required for improvement. ■ Arrange immediate on-site supervision. ■ Check staff competence. ■ Consider intensive on-the-job training to remedy weaknesses. ■ Check reagent quality and the microscope. ■ Conduct regular follow-up for corrective action.

ANNEX 5. EXAMPLE CHECKLIST FOR INTERNAL QUALITY ASSURANCE

Category	Statement	Yes	No
Human resources	The staff who perform malaria diagnosis are qualified. They have completed a refresher course or a malaria microscopy competence assessment during the past 2 years.		
Documents	There are current SOPs for malaria microscopy. There are current bench aids for malaria microscopy.		
Laboratory design	There is sufficient working space for each laboratory staff member.		
	The electric microscope(s) faces a blank wall (is not located directly in front of a window).		
	The laboratory has access to a clean water supply.		
	The laboratory has a regular supply of electricity during working hours. There is a back-up generator.		
	There are hand-washing facilities.		
	There is good ambient lighting at all times (including during cloudy weather).		
	There is an adequate electrical supply for the microscope(s).		
	There is adequate storage space for reagents, equipment and storage of slides. There are enough slides boxes.		
	There is a safe waste management system.		
	Laboratory chairs or stools are suitable for microscopy.		
Equipment maintenance	There is a regular microscope maintenance system. Microscope maintenance is documented in the laboratory maintenance register.		
Quality of the microscope	The microscope(s) is binocular and electrically powered.		
	The microscope lamp(s) has sufficient power to provide effective illumination at small aperture settings.		
	The light source can be centred.		
	The microscope(s) has plan C x100 objectives.		

Category	Statement	Yes	No
	Blood films can be brought into sharp focus at x100 oil immersion magnification.		
	There are enough spare bulbs.		
	The stage movement mechanism is precise and stable.		
Microscope slides	Microscope slides are clean.		
	Microscope slides are not oily to the touch.		
	Microscope slides do not have scratches on the surface. aberrations.		
	Microscope slides do not give a blue background (at x100) after staining.		
	Microscope slides do not have fungal contamination.		
	Slides are protected against fungal contamination (in high humidity).		
	Microscopes slides are protected against dirt and flies.		
Methanol	Methanol is of analytical reagent grade.		
	Methanol is supplied to the laboratory in the original sealed container from the manufacturer and is not repackaged by the supplier.		
	Methanol is not oily. (If you place some methanol on your fingers, it is not sticky.)		
	RBCs in the thin blood film are not deformed or blistered due to poor-quality methanol.		
	The methanol used for slide fixing is stored in moisture-proof containers.		
Giemsa stain	Only stain prepared from high-quality Giemsa powder is used.		
	Commercial Giemsa stain is supplied to the laboratory in the original sealed container.		
	The stain is within the manufacturer's expiry date		
	The laboratory has a stain QC register of the batch numbers and expiry dates of supplies received, the QC results on each batch (staining time, staining quality, optimal pH of use) and any problems encountered.		
	Unstained slides are kept for testing new batches of stain for Giemsa QC.		
	Stock stain is stored in a tightly sealed dark–glass bottles.		
	Stock stain is not stored in direct sunlight or near a heat source.		
	The stock stain used by the laboratory was prepared < 2 years previously.		
	Stained blood films do not contain stain precipitate.		

Category	Statement	Yes	No
Diluted Giemsa stain	Stock stain is always diluted in buffer to the correct pH.		
	The diluted stain contains no stain precipitate.		
	The surface of the diluted stain does not have an oily appearance. For horizontal slide staining (on a staining rack), this is best observed after the stain has been added to the slides. It can be due to use of poor-quality methanol for preparing Giemsa stain from powder.		
	Diluted stain is always discarded within 30 min of preparation.		
Thick blood films	More than 95% of thick films are of the correct thickness. Newsprint can just be read through the thick film while it is still wet, and RBC are completely lysed.		
	Less than 2% of thick films show flaking at the centre of the smear (a hole in the centre of the thick film).		
	100% of the thick films are correctly stained.		
	None of the thick films are contaminated with stain precipitate.		
	There is a protocol for preparation of thick films of the correct thickness from patients with severe anaemia.		
	Slide warmers are used with caution in conditions of high humidity.		
	Thick blood films are approximately 1 cm in diameter.		
Thin blood films	More than 95% of thin films have a smooth, semi-circular tail.		
	Thin and thick blood films are made on the same slides and are correctly labelled.		
	In > 95% of thin films, RBC are just touching and do not overlap on 20–30% of the film (the examination area, or area of ideal thickness).		
	No thin films have water damage (retractile artefacts inside RBC).		
	Thin films are fixed in 2 s immediately after drying. Precautions are taken to avoid fixing the thick film.		
Staining	The laboratory has a pH meter that reads to two decimal places.		
	The pH meter is calibrated with calibration buffers according to the manufacturer's instructions.		
	A pH-adjusted buffer is always used to prepare diluted Giemsa.		

Category	Statement	Yes	No
	The pH of the buffer is calibrated for each batch of Giemsa.		
	Slides are always washed in water of the same pH as the buffer used for diluting Giemsa.		
	Diluted Giemsa is always prepared in a clean measuring cylinder.		
	There is an absolute rule that diluted Giemsa stain be discarded < 30 min after preparation.		
	The trophozoite chromatin stains red to “rusty red”.		
	The trophozoite cytoplasm stains blue to strong blue.		
	The thick film background stains light-pink to grey.		
	The RBC in the thin film stain grey–blue.		
	The nuclear lobes of the polymorphs stain significantly darker than the cytoplasm.		
	Slides are always washed from the thin film end.		
	All slides are washed gently by a technique in which stain “floats” off without disturbing the thick film.		
	Laboratory staff who perform staining wear protective clothing to protect their personal clothing.		
Counting	The laboratory reports the actual number of parasites, when required, against 500 (200) WBC, according to the WHO-recommended method.		
	Calibrated tally counters for counting parasites and WBC separately are available.		
Slide reading time	All laboratory staff who report malaria examination results read a minimum of 10 thick blood films each month.		
	Laboratory staff always examine a minimum of 100 fields before reporting “No malaria parasites seen”.		
	Microscopists are not under pressure to examine slides more quickly than the standard reading time (such as at the end of the day or in “urgent” cases).		
	A laboratory protocol ensures that microscopists do not examine malaria slides continuously for more than 2 h without a 15 min break.		
Species identification	Thin films are available for species identification when a mixed infection is suspected or species identity is unclear on the thick film.		

Category	Statement	Yes	No
Reporting	Results are reported within a maximum of 1 h 30 min.		
	All slide results are correctly reported in the laboratory register after examination is completed, with slide identification, parasite detection result, species if positive and parasite counting.		

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