Malaria Microscopy Quality Assurance MANUAL
Version 1
Malaria Microscopy Quality Assurance Manual - Version 1

1. Malaria -- diagnosis. 2. Microscopy -- standards. 3. Quality control

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Table of Contents

Acknowledgement vii
Executive Summary ix
Preface xi
Glossary Of Terms xii
Defining Quality Assurance xvii
Abbreviations xix
1. The Need For Improving Quality Assurance Of Malaria Microscopy 1
   1.1 The need for accurate diagnosis 1
   1.2 The role of light microscopy in malaria control today 2
   1.3 Current limitations of microscopy of malaria 3
   1.4 Improving competency and performance 3
       1.4.1 Defining competency and performance 4
       1.4.2 Assessing the performance of malaria microscopy 5
2. The Structure And Function Of The Quality Assurance Programme 7
   2.1 Scaling up of quality assurance programmes 8
   2.2 The basic structure 8
       2.2.1 Central level National Reference Group 9
       2.2.2 Intermediate/provincial level 11
       2.2.3 District health centre (township/village) level 12
   2.3 QA programme manager 12
       2.3.1 Selection 12
       2.3.2 Responsibilities 12
   2.4 Essential functional elements of the programme 13
   2.5 Tasks to be carried out at each level 13
       2.5.1 Malaria diagnosis 13
       2.5.2 Quality assurance 14
   2.6 The role of clinical staff in quality assurance 15
3. The Plan Of Action 17
   3.1 Goals and objectives 18
   3.2 Essential elements 19
   3.3 Pre-implementation assessment 19
   3.4 Workload 22
   3.5 Costing of quality assurance programmes 25
       3.5.1 Initial costs 25
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5.2</td>
<td>Recurrent costs</td>
<td>25</td>
</tr>
<tr>
<td>4.</td>
<td>Supplies And Equipment</td>
<td>27</td>
</tr>
<tr>
<td>4.1</td>
<td>Standardized lists</td>
<td>28</td>
</tr>
<tr>
<td>4.2</td>
<td>Establishment of a supply chain</td>
<td>28</td>
</tr>
<tr>
<td>4.3</td>
<td>Microscopes</td>
<td>29</td>
</tr>
<tr>
<td>4.4</td>
<td>Microscope slides</td>
<td>29</td>
</tr>
<tr>
<td>4.5</td>
<td>Stains</td>
<td>31</td>
</tr>
<tr>
<td>5.</td>
<td>Establishing A National Core Group Of Expert Microscopists</td>
<td>32</td>
</tr>
<tr>
<td>5.1</td>
<td>Aims of accreditation</td>
<td>33</td>
</tr>
<tr>
<td>5.2</td>
<td>Principles of accreditation</td>
<td>33</td>
</tr>
<tr>
<td>5.3</td>
<td>Planning accreditation courses</td>
<td>33</td>
</tr>
<tr>
<td>5.3.1</td>
<td>The aim</td>
<td>33</td>
</tr>
<tr>
<td>5.3.2</td>
<td>The trainer/facilitator</td>
<td>34</td>
</tr>
<tr>
<td>5.3.3</td>
<td>Syllabus and timetable</td>
<td>34</td>
</tr>
<tr>
<td>5.3.4</td>
<td>Requirements for equipment and supplies</td>
<td>36</td>
</tr>
<tr>
<td>5.3.5</td>
<td>Blood slides</td>
<td>36</td>
</tr>
<tr>
<td>5.3.6</td>
<td>Standardized operating procedures</td>
<td>37</td>
</tr>
<tr>
<td>5.4</td>
<td>Basic elements of assessment</td>
<td>38</td>
</tr>
<tr>
<td>5.4.1</td>
<td>Conditions for assessment</td>
<td>38</td>
</tr>
<tr>
<td>5.4.2</td>
<td>Pre-course assessment</td>
<td>38</td>
</tr>
<tr>
<td>5.4.3</td>
<td>Final assessment</td>
<td>38</td>
</tr>
<tr>
<td>5.5</td>
<td>Minimum grades for expert accreditation</td>
<td>39</td>
</tr>
<tr>
<td>6.</td>
<td>Training Of Basic Level Microscopists</td>
<td>41</td>
</tr>
<tr>
<td>6.1</td>
<td>Selection of basic level microscopists</td>
<td>42</td>
</tr>
<tr>
<td>6.2</td>
<td>Training courses for of basic level microscopists</td>
<td>42</td>
</tr>
<tr>
<td>6.2.1</td>
<td>WHO Training Manuals</td>
<td>42</td>
</tr>
<tr>
<td>6.2.2</td>
<td>Training objectives</td>
<td>43</td>
</tr>
<tr>
<td>6.2.3</td>
<td>Assessing competency</td>
<td>45</td>
</tr>
<tr>
<td>6.2.4</td>
<td>Minimum grades for competency</td>
<td>45</td>
</tr>
<tr>
<td>6.3</td>
<td>Refresher training</td>
<td>46</td>
</tr>
<tr>
<td>6.4</td>
<td>Retraining</td>
<td>46</td>
</tr>
<tr>
<td>7.</td>
<td>Supervision Of Malaria Microscopy Facilities</td>
<td>47</td>
</tr>
<tr>
<td>7.1</td>
<td>Planning the monitoring process</td>
<td>48</td>
</tr>
<tr>
<td>7.2</td>
<td>Supervisory visits (on-site evaluations)</td>
<td>50</td>
</tr>
<tr>
<td>7.2.1</td>
<td>The need</td>
<td>50</td>
</tr>
<tr>
<td>7.2.2</td>
<td>Frequency of visits</td>
<td>51</td>
</tr>
<tr>
<td>7.2.3</td>
<td>Expected outcomes</td>
<td>51</td>
</tr>
<tr>
<td>7.2.4</td>
<td>Check list of tasks to be carried out</td>
<td>51</td>
</tr>
<tr>
<td>8.</td>
<td>Validation Of Malaria Slide Results (Cross-checking)</td>
<td>53</td>
</tr>
<tr>
<td>8.1</td>
<td>Requirements for cross-checking</td>
<td>54</td>
</tr>
<tr>
<td>8.2</td>
<td>Principles of cross-checking and classification of errors</td>
<td>55</td>
</tr>
<tr>
<td>8.3</td>
<td>The protocol for slide validation</td>
<td>59</td>
</tr>
</tbody>
</table>
8.3.1 Overall objectives 59
8.3.2 Scope 60
8.3.3 Slide storage 60
8.3.4 Sample selection from laboratory register 60
8.3.5 Sample size 60
8.3.6 Sample selection criteria 60
8.3.7 Cross-checking 62
8.3.8 Recording of results 63
8.3.9 Statistical analysis 64
8.3.10 Reporting of results 68
8.4 Action to be taken case of discordant results 68

9. Reference Training Slide Sets 69
9.1 The need 70
9.2 Constitution of a national slide bank 70
9.3 The aims 70
9.4 Plan of action 71
9.5 Costing 73
9.6 Selection of staff 73
9.7 Methods for slide collection 74
  9.7.1 Hospital/health centre 74
  9.7.2 Selective surveys 74
9.8 Selection of donors 75
9.9 Ethical considerations 75
9.10 Data management 76
9.11 Slide bank storage and maintenance 77

10. Self Monitoring Of Laboratory Procedures (Internal Quality Assurance) 79
ANNEX 1 Model list for equipment and supplies for peripheral malaria laboratory 81
ANNEX 2 Model list of equipment and supplies for an assessment course for expert microscopists. 84
ANNEX 3 Model theory test for expert malaria microscopists 86
ANNEX 4 Check list and reporting form for supervisory visits 87
ANNEX 5 The rationale and principles of the recommended method for the selection of slides for cross-checking 94
ANNEX 6 Model reporting form for cross-checking malaria blood slides: no species identification 97
ANNEX 7 Model reporting form for cross-checking malaria blood slides: species identification 98
ANNEX 8 Standard operating procedure for establishing a national reference slide bank 99
ANNEX 9 Model information sheet and consent form for slide bank donors 118
ANNEX 10 Model data sheet for slide bank donors 121
ANNEX 11 Template for making thick films 122
ANNEX 12 Check list for internal quality assurance 123
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MSF   Médecins Sans Frontières
AMI   Australian Army Malaria Institute
KEMRI   Kenya Medical Research Institute
WRAIR   Walter Reed Army Institute of Research
AMREF   African Medical and Research Foundation
NICD   National Institute for Communicable Diseases
RITM   Research Institute for Tropical Medicine
ACTMalaria  Asian Collaborative Training Network for Malaria
US CDC   United States Centers for Disease Control and Prevention

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The manual is a consensus document, and does not imply the individual opinions of any individual author or contributor.
Early diagnosis and prompt, effective treatment is the basis for the management of malaria and key to reducing malaria mortality and morbidity. Demonstration of the presence of malaria parasites prior to treatment with antimalarial drugs is fundamental to this goal, as clinical diagnosis has poor accuracy and leads to over-diagnosis of malaria with resultant poor management of non-malarial febrile illness and wastage of 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 for ensuring good health outcomes and optimal use of resources.

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

The primary aim of malaria microscopy QA programmes is to ensure that microscopy services are manned by competent and motivated staff, supported by effective training and supervision that maintains a high level of staff competency and performance and by a logistics system that provides and maintains an adequate supply of reagents and equipment.

QA programmes must be:

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

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

This manual is designed primarily to assist managers of national malaria control programmes and laboratory services to develop and maintain a sustainable malaria
malaria microscopy QA programme. It outlines a hierarchical structure based on re-training, validation, and the development of competency standards designed to ensure the quality of diagnosis necessary for a successful malaria programme, while remaining within the financial and personnel resources likely to be available. Without an efficient QA programme, resources spent on diagnostic services are likely to be wasted and clinicians will have no confidence in the results.

The mode of implementation of the QA system outlined in this manual will vary according to the organization of the national laboratory services dealing with malaria, which may fall under the national malaria control programme, or under a separate laboratory structure working closely with the malaria programme. The microscopists may be tertiary trained laboratory technicians performing a range of specialized diagnostic activities, or health workers trained specifically in malaria microscopy without wider laboratory roles. In either case, the principles remain the same.

At a minimum, a malaria microscopy QA programme should include the following.

- A central coordinator(s) to oversee QA.
- A reference (core) group of microscopists at the head of a hierarchical structure, supported by an external QA programme and with demonstrable expertise in overseeing programme training and validation standards.
- Good initial training with competency standards that must be met by trainees prior to operating in a clinical setting.
- Regular retraining and assessment/grading of competency, supported by a well-validated reference slide set (slide bank).
- A sustainable cross-checking (validation) system that detects gross inadequacies without overwhelming validators higher up the structure, with good feedback of results and a system to address inadequate performance.
- Good supervision at all levels.
- Good logistical management, including supply of consumables and maintenance of microscopes.
- Clear standard operating procedures (SOPs) at all levels of the system.
- An adequate budget is required as an essential part of funding for malaria case management.

The manual describes the essential elements necessary to put this structure in place.
Executive Summary
Preface

This manual is based on the recommendations of a series of informal consultations organized by the World Health Organization on the subject, particularly a biregional meeting by the WHO Regional Offices for the Western Pacific and South-East Asia from 18 to 21 April 2005 in Kuala Lumpur, Malaysia. Another meeting was held on 3 March 2006 in Geneva, Switzerland. An informal consultation took place in Geneva from 7 to 8 February 2008, as well as extensive consultations with international malaria experts.

The manual is designed primarily to assist managers of national malaria control programmes and general laboratory services who are responsible for malaria control. It should also be of interest to those non-governmental organizations and funding agencies that are involved in the support of malaria disease management and malaria diagnosis in particular.

It is not designed for the quality assurance of microscopy used in research situations, such as clinical trials of new drugs and vaccines and the monitoring of parasite drug resistance.

It forms part of a series of WHO documents that are designed to assist countries in improving the quality of malaria diagnosis. These include the revised WHO Training Manuals, Basic Malaria Microscopy Part I. Learner’s Guide and Basic Malaria Microscopy Part II. Tutor’s Guide and the revised WHO Bench Aids for the diagnosis of malaria infections.

Note on the use of the term ‘Microscopist’

Malaria programmes in different regions use various terms to denote a person who uses a microscope to read blood films to aid or confirm the diagnosis of malaria and report on their findings. This task may be performed in many contexts, including case management in a small rural clinic, as part of a teaching curriculum in a university, or to provide a reference standard in a large clinical trial. It may form just one of the duties of a senior laboratory consultant or technician in a reference laboratory, or the entire workload of a staff member in a small outpatient clinic. In this manual, the term ‘microscopist’ is used to denote any person who carries out such an activity, as the principles discussed in the manual apply, to differing degrees, to personnel performing these tasks at all levels of malaria diagnostic programmes.
Glossary of Terms

Administrative levels of the laboratory services
Laboratory services are typically organized into three main levels: the national or central level, regional/provincial or intermediate level, and the district/health centre or peripheral level. Laboratory services at the national level might be an integral part of the national malaria control programme, of the laboratory services of the general health services or a suitably designated national reference laboratory. The peripheral level of the laboratory services may comprise a health worker within the village or the community, primary diagnostic facilities at small fixed sites that deal with outpatients or secondary diagnostic laboratories within a hospital or health posts which deal with both in- and out-patients.

Artemisinin-based combination therapies (ACTs)
Combination therapy with antimalarial drugs is the simultaneous use of two or more antimicrobial drugs with independent modes of action and different biochemical targets in the parasite to improve therapeutic efficacy and delay the development of resistance to the individual components. The World Health Organization's current recommendations on the use of ACTs are based on four combinations: artesunate-amodiaquine, artemether-lumefantrine, artesunate-mefloquine, and artesunate plus sulfadoxine-pyrimethamine.

Benchmarking
A comparison of the performance of all laboratories and/or test centres within a programme using standardized indicators, e.g. comparing the relative performance of laboratories in a quality control programme.

External quality assurance
A system whereby a reference laboratory sends stained blood films to a laboratory for examination. The laboratory receiving the slides is not informed of the correct result of the slides until the laboratory has reported their findings back to the reference laboratory.

False negative slide
A positive smear that is misread as negative.

False positive slide
A negative smear that is misread as positive.

Feedback
The process of communicating results of external quality control to the original laboratory, including identification of errors and recommendations for remedial action.

First-line and second-line antimalarial drugs
First-line drugs are those routinely used by a national malaria control programme to treat uncomplicated malaria disease. Second-line drugs are those used to treat treatment failures that occur with the use of first-line drugs.
Global Fund to Fight AIDS, Tuberculosis and Malaria
The Global Fund was created by the United Nations as a channel for substantial additional resources for the control of AIDS, tuberculosis and malaria required to meet the United Nations Millennium Development Goals.

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

National Malaria Control Programme
The countrywide permanent programme responsible for all activities related to controlling malaria. These include integrated efforts with general health services to provide diagnosis and treatment for malaria.

National Reference or Central Laboratory
This may exist as part of the central public health laboratory, the national malaria control programme or as a governmentally approved facility within academia. It plays an essential role in the organization and maintenance of the network of laboratories for malaria diagnosis including the development of guidelines for standardizing methodology, maintenance of slide banks, production of locally adapted training materials, overseeing training activities, assuring quality of testing and supporting external QA in collaboration with the national malaria control programme.

Performance standard
A level of performance that is considered acceptable and which all laboratories and/or test centres should meet or exceed. Setting performance standards allows the identification of laboratories that are not performing satisfactorily.

Quality assurance
The monitoring and maintenance of high accuracy, reliability and efficiency of laboratory services. Quality assurance addresses all factors that affect laboratory performance including test performance (quality control, internal and external) equipment and reagent quality, workload, workplace conditions, training and laboratory staff support.

Quality control
Measures the quality of a test or a reagent. For malaria microscopy, the most common form of quality control (QC) is the cross-checking of routine blood slides to monitor the accuracy of examination. Quality control also encompasses external quality control and reagent quality control. Cross-checking QC is a system whereby a sample of routine blood slides are cross-checked for accuracy by a supervisor or the regional/national laboratory. Reagent QC is a system of formally monitoring the quality of the reagents used in the laboratory.
Rapid Diagnostic Tests
Rapid Diagnostic Tests (RDTs) are immuno-chromatographic tests that detect parasite specific antigens in a finger-prick blood sample. Some tests detect only one species (*Plasmodium falciparum*), others detect one or more of the other three species of human malaria parasites (*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 slides, detected by microscopy, among all those examined within a laboratory over a defined period of time.

**SMART indicators**
Indicators of performance that are Specific, Measurable, Achievable, Attainable, Realistic and Timely.
The quality assurance (QA) of a malaria laboratory or diagnostic programme is a system designed to continuously and systematically improve the efficiency, cost-effectiveness and accuracy of test results. It is critical that QA ensure:

- the clinical teams have full confidence in the laboratory results
- the diagnostic results are of benefit to the patient and the community.

These demands can only be met through a commitment to QA that ensures the microscopic services are staffed by competent and motivated staff supported by both effective training and supervision and a logistics system that provides an adequate and continual supply of quality reagents and essential equipment which are maintained in working order.

The principles and concepts of QA for microscopic diagnosis of malaria are similar to those for microscopic diagnosis of other communicable diseases, such as other protozoan diseases, tuberculosis and helminth infections. This provides a potential for the integration of laboratory services where it is feasible and cost-effective.
### Abbreviations

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<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACT</td>
<td>Artemisinin-based Combination Therapy</td>
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<td>ACTMalaria</td>
<td>Asian Collaborative Training Network for Malaria</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<td>EQA</td>
<td>External Quality Assurance</td>
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<td>GFATM</td>
<td>Global Fund to Fight AIDS, Tuberculosis and Malaria</td>
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<td>IRS</td>
<td>Indoor residual spraying</td>
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<td>JSB</td>
<td>Jaswant Singh Battacharya stain</td>
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<td>LQAS</td>
<td>Lot Quality Assurance System</td>
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<td>MSF</td>
<td>Médecines Sans Frontières</td>
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<tr>
<td>NGO</td>
<td>Nongovernmental organization</td>
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<td>NMCP</td>
<td>National Malaria Control Programme</td>
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<td>NRL</td>
<td>National Reference Laboratory</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>QA</td>
<td>Quality assurance</td>
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<td>QC</td>
<td>Quality control</td>
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<td>RBC</td>
<td>Red blood cell</td>
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<td>RCC</td>
<td>Red blood cell count</td>
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<td>RDT</td>
<td>Rapid diagnostic test</td>
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<td>SOP</td>
<td>Standard operating procedure</td>
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<td>SPR</td>
<td>Slide positivity rate</td>
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<tr>
<td>WBC</td>
<td>White blood cell</td>
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<tr>
<td>WCC</td>
<td>White blood cell count</td>
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<td>WHO</td>
<td>World Health Organization</td>
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Chapter 1

The Need for QA of Malaria Microscopy

The detection of malaria parasites by light microscopy is still the primary method of malaria diagnosis in health clinics and hospitals throughout the world. This requires a reliable microscopic service that:

- is cost-effective
- is accurate and timely
- has 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 at all levels.

1.1 The need for accurate diagnosis

The first suspicion of malaria is usually based on clinical criteria and, in many situations, symptom-based diagnosis is the sole basis for treatment in areas where malaria is endemic. Even in areas of high transmission, most cases of fever are usually not malaria. This results in a high rate of over treatment with antimalarial drugs as the clinical diagnosis of malaria is very non-specific. While this might have been acceptable in the past when malaria was treatable with low cost drugs, it is not acceptable today.

A diagnosis based on clinical symptoms alone has very low specificity, with the result that the number of false-positive results can be considerable and other diseases may be overlooked and not treated in a timely manner. This contributes to an increase in non-malaria morbidity and mortality, the misuse of antimalarial drugs, the development of parasite drug resistance, increased costs to the health services and patient dissatisfaction.

An accurate, correct laboratory diagnosis is essential as false negatives can result in untreated malaria patients and potentially severe consequences, including death. False negatives can also significantly undermine both clinical confidence in laboratory results and credibility in the community. False-positive results are equally problematic. Patients presenting with fever not caused by malaria may be misdiagnosed and the true cause of their fever not treated. This can also have severe consequences, including the death of the patient. In addition, misdiagnosis of malaria will result in the unnecessary prescription of high cost drugs and the unnecessary exposure of the patient to potentially toxic drugs. This is a needless burden to both the patient and the medical services.

The need and the importance of accurate microscopic diagnosis for malaria has become acute with the spread of antimalarial drug resistance and of multidrug-resistant
*Plasmodium falciparum*, in particular. As a response to antimalarial drug resistance, WHO now recommends that first-line treatment for *falciparum* malaria in all countries experiencing resistance to conventional monotherapies, such as chloroquine, sulfadoxine/pyrimethamine and amodiaquine, should be combination therapies, preferably those containing an artemisinin derivative, known as artemisinin-based combination therapies (ACTs). It is now considered that an effective first-line antimalarial treatment would have a greater impact on reducing mortality than merely improving either the second-line treatment or the management of severe malaria. Consequently, ACTs are now increasingly used worldwide.

Artemisinin-based combination therapies cost far more than chloroquine, amodiaquine or sulfadoxine/pyrimethamine and improving the accuracy of malaria diagnosis means that ACT treatment is targeted only to those who need this type of therapy, which will reduce the current over-consumption of antimalarial drugs. This reduces costs to health services and has a beneficial effect on implementing drug policies based on combination therapies. The need for extending laboratory diagnostic services to the periphery of the health services is, therefore, self-evident.

Good clinical practice dictates that the presence of parasites must be confirmed by a laboratory diagnosis in most epidemiological situations. However, if this is not logistically possible for all suspected cases of malaria, it is particularly desirable that the presence of parasites is confirmed in all suspected cases of treatment failures and severe disease as well as for diagnosing uncomplicated malaria during low transmission seasons.

### 1.2 The role of light microscopy in current malaria control practice

A laboratory confirmatory diagnosis is recommended in patients of all age groups in areas of low and moderate transmission and in children of >5 years and adults in areas of high transmission, the possible exception being children of <5 years in areas of high transmission.

Laboratory diagnosis by microscopic examination of stained blood films continues to be the method of choice, or the common reference standard, for case management and epidemiological studies. RDTs are also an important component of a diagnostic strategy for malaria and can be used to confirm the presence of parasites in certain circumstances. They cannot be considered as a gold standard.

Light microscopy is also essential for parasite diagnosis during clinical and field trials of antimalarial drugs and vaccines and for the QA of other forms of malaria diagnosis, such as RDTs.

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1. The use of ACTs is based on the advantages of artemisinin derivatives which are: rapid reduction of parasite densities, rapid resolution of clinical symptoms, effective action against multidrug resistant *falciparum* malaria, few clinical adverse reactions and reduction of gametocyte carrier rates which may reduce transmission.

2. WHO has produced the following documents to assist countries in evaluating and using RDTs: *Making Rapid Diagnosis Work, Methods Manual for Laboratory Testing of Malaria Rapid Diagnostic Tests, and Assessing RDT Cost-Effectiveness* (an Interactive Microsoft Excel-based Programme).
Microscopic diagnosis has many advantages, for example, it:

- has low direct costs if the infrastructure to maintain the service is already available;
- is sensitive if the quality of microscopy is high;
- can differentiate between malaria species;
- can determine parasite densities; and
- can also be used to diagnose other diseases.

1.3 Current limitations of microscopic diagnosis of malaria

It has been difficult to maintain good quality microscopy especially at the periphery of the health services, where most patients are treated in spite of the fact that the importance of light microscopy is well recognized.

The current limitations of malaria microscopy are well recognized and documented, including the:

- lack of political commitment to support the development and expansion of laboratory services;
- lack of funds to support the integration of malaria diagnosis into the general laboratory services;
- poor quality of microscopy, particularly at the peripheral level;
- difficulties in maintaining microscopy facilities in good order;
- logistic problems and high costs of maintaining adequate supplies and equipment;
- lack of adequate training and retraining of laboratory staff;
- delays in providing results to clinical staff;
- lack of QA and supervision of laboratory services; and
- inability to cope with the workload of traditional systems for cross-checking of routinely taken malaria slides, often due to inadequate human and financial resources.

These limitations can only be overcome by new health policies that acknowledge the importance of strengthening laboratory services, the need for adequate funding, and the implementation of a QA system which ensures that:

- there is constant training, supervision of staff and quality control of their tasks;
- the structure of the programme is practical and sustainable with adequate staff and resources;
- slide collection, staining and reading are accurate, timely and linked to clinical diagnosis;
- results are quickly provided to clinicians;
- clinicians can trust the results; and
- there is logistic support to provide quality supplies and equipment.

1.4 Improving competency and performance

In many endemic countries, malaria microscopists receive initial training and are assumed to be competent for the remainder of their careers. Attendance at refresher
courses and more advanced training are seen as a reward rather than a continuation of their education. In some countries malaria microscopists do not even receive formal training and are expected to learn from others how to do their job, a situation no longer acceptable.

A high level of competency and performance can only be achieved if microscopists at all levels are supported by a training and assessment programme that is continuous, allows refresher training when required, is linked to career advancement for those who are high performers and is developed according to international standards. Although these standards are primarily intended for national programme staff and trainers, the same standards should also be applicable to staff working with nongovernmental organizations (NGOs) and in the private sector. Countries should set standards to ensure that the experience of each participant enrolled in a training course is appropriate.

It is recommended that, where QA programmes for malaria microscopy are not adequately developed, priority should be given to the training and assessment of senior microscopists at the central and intermediate levels since these are the individuals who will be responsible for the training and assessment of peripheral staff.

1.4.1 Defining competency and performance

**Competency** in microscopy is the ability of a microscopist to perform an accurate examination and correct reporting of a malaria blood film.

Measuring competency requires:

- a definition of the specific skills that are required at each level of the QA system;
- setting standards of competency;
- a definition of the minimum educational requirements for training in microscopy;
- standardized training materials and courses; and
- standardized assessments at the end of training.

**Performance** in microscopy is the ability of the microscopist to perform and report accurately malaria slide examinations in routine practice.

Measuring the performance of a microscopist requires the:

- clear definition of performance standards;
- standardized and unbiased cross-checking of a sample of slides routinely examined by the microscopist; and
- monitoring of performance.

Performance can be improved by:

- providing good quality microscopes, stains and supplies;
- ensuring a managed and reasonable workload;
- an effective response to problems by both supervisors and microscopists;
- consultation visits by supervisors;
- retraining, when appropriate; and
- increasing motivation through personal certification of all supervisors and microscopists, linked to a career structure with associated financial incentives.

### 1.4.2 Assessing the performance of malaria microscopy

The performance of malaria microscopy must be continuously monitored by a quality assurance programme based on pre-defined standards. QA includes two essential components:

- Assessment of the accuracy of the examination of thick and thin films for malaria diagnosis and monitoring. This may be performed by visits from supervisors and/or external blinded cross-checking of slides.
- Monitoring systems for staff competence, equipment, reagents, stock control, workload, registration and reporting.

The primary aim of QA programmes at the basic level will be the identification of laboratories with serious problems resulting in poor performance. However, the ultimate goal is to assure the performance of all laboratories and test centres performing malaria diagnostics. This needs to be incorporated into medium-term planning for programmes starting from a low baseline. Programmes with a more developed infrastructure should use a system of QA as comprehensive as possible. For all programmes, the goal is to establish a regional or national benchmark QC system for blinded cross-checking of slides, and central reporting of QA indicators for at least equipment and reagent performance, stock control and workload. Further, national or regional programmes should develop minimum acceptable standards for these parameters. The relationship between competence and performance is illustrated in Figure 1.

**Figure 1 Ensuring and demonstrating good performance in malaria microscopy**
Chapter 2
The Structure And Function Of Quality Assurance Programmes

Fig. 2 Structure and function of the quality assurance system

QA programmes must be:
- realistic, feasible and sustainable
- compatible with the different situations and needs of each country
- a catalyst for change to a culture of quality
- able to promote the best quality in the prevailing circumstances
2.1 Scaling-up of quality assurance programmes

Scaling-up of malaria QA has become a priority as more effective intervention methods lead to a reduction in malaria prevalence in many areas and a resultant increased need to distinguish malarial from non-malarial fevers. The increasing use of high-cost antimalarial drugs also makes parasite-based diagnosis imperative. In many countries, QA programmes need to be rebuilt after many years of neglect and/or under funding. This rebuilding cannot be achieved without increased investments in financial and human resources. Some countries may be able to provide these resources nationally, but many others will require external assistance from the international community.

Regardless of the source of these new investments, national programmes will need to develop realistic proposals with credible budgets that demonstrate value for money to convince decision-makers that there are benefits to be gained by investing in rebuilding the infrastructures and human resources required to ensure quality malaria microscopy. The importance of malaria QA should be linked to cost savings, as well as case management, showing that improved QA will save costs, lives and reduce morbidity from other diseases.

The rebuilding effort will need to be based on a phased plan of action covering a period of at least five years and take into consideration the Millennium Development Goals set for 2015.3

2.2 The basic structure of QA programmes

WHO has recommended for many years that the integration of malaria microscopy and its QA with that for other microscopically diagnosed communicable diseases, where such programmes are compatible and based on similar elements in the health service.

Thus, in those countries where malaria microscopy is carried out by the general health services, the development of the malaria QA programme should be the responsibility of the national laboratory services with the technical support in the National Malaria Control Programme (NMCP) and in collaboration with other institutions in the country carrying out QA, such as universities and NGOs. Such a system will:

- simplify the administration, logistics of supply of reagents and equipment, reporting and evaluation of the performance of microscopy;
- be less resource-intensive where QA for malaria could “piggy back” on other QA schemes by using existing resources and infrastructures;
- result in the improvement of other laboratory sectors, including the use of new tests and the supply chain for reagents and equipment as well as the maintenance of microscopes and other equipment in working order;
- result in the optimal use of microscopes and other equipment in laboratory with low workloads;
- maintain general proficiency when there is a low workload;

3 see <http://www.un.org/millenniumgoals/>
be more interesting for the laboratory technicians and therefore increase staff commitment;
- provide the same approach and grading for measuring the competence and performance of microscopists, making it easier to implement a career structure;
- require a single budget;
- be easier to monitor and evaluate, resulting in a more transparent system; and
- be attractive to donors.

In those countries where the general health services do not have the capability, at present, to develop a malaria microscopy QA programme, the NMCP should take the responsibility for its development in collaboration with general health services and other interested partners, with the long-term goal of integrating malaria QA into general health services when conditions are favourable.

The common hierarchical organization of general laboratory services into national (central), provincial (intermediate) and district/health centre (peripheral) laboratories is ideal for the management and operation of the QA system. The corresponding elevated standards and responsibilities at each level also have the potential for the development of a career structure for microscopists. Such a career structure is considered extremely important since it will make microscopy more attractive for those entering the service and give incentives for those already in service. The relationship of this structure to the functions at the different levels is shown in the diagram on the opening page of this chapter.

### 2.2.1 Central level (National Reference Group)

The central level plays a key role in the delivery of diagnostic services at all levels, as well as being responsible for the planning, implementation and monitoring of QA. Ideally, this should be a laboratory within the general laboratory services of the Ministry of Health. Such a laboratory may be associated with a large hospital or a research institute but if these services are unable to coordinate and carry out malaria QA, it could be within the NMCP. Whatever the arrangement, it is therefore important that a competent laboratory is designated as the National Reference Laboratory (NRL) and that the NMCP closely collaborates and coordinates with it. It is advantageous if the NRL is located close to the offices of both the national laboratory services and the malaria control programme.

The NRL should participate in an international QA programme that includes recognition and accreditation of expertise among its staff. Such retraining and accreditation is essential to ensure expertise, and to demonstrate the expertise of the NRL for training and validation purposes within the national QA system. These programmes are discussed in Chapter 6.

The NRL should be responsible for establishing national standards for:

- training courses;
- preparation/adaptation of training materials according to local situations and languages;
- assessment of competency and performance of microscopists according to international standards;
- accreditation of microscopists; and
- laboratory procedures and equipment.

This centre could also be the focal point for international contacts and should strive for international and regional recognition as a centre of excellence.

All staff of the NRL should have appropriate training and experience, and have demonstrable commitment to high standards of scientific practice and laboratory management.
2.2.2 Intermediate/provincial level

Laboratory technologists at this level should be responsible for the supervision and monitoring of activities to maintain the quality of the district and peripheral laboratories. They would need to carry out the external cross-checking of slides taken at district/health centre levels (peripheral level) and be responsible for:

- The feedback of results;
- The planning and implementation of training and retraining activities; and
- Ensuring that equipment is maintained in good working order and that there are no breakdowns in the supply-chain.
2.2.3 District/health centre (township/village) level

Depending on the country, the organization of laboratory services at the peripheral level may be at:

- **village or community level**—where tests are carried out by village volunteers, mobile health teams or health posts attached to peripheral clinics/hospitals;
- **primary diagnostic facilities**—small fixed site health centre dealing mainly with outpatients; and
- **secondary diagnostic facilities**—such as laboratories within hospitals that deal with both inpatients and outpatients.
2.3 QA programme manager

Effective management by trained and competent senior staff will be essential to the development and success of all QA programmes.

2.3.1 Selection

A national focal point should be appointed with a clear mandate to oversee the implementation of the QA programme. This national QA co-coordinator or manager should be a senior laboratory technologist/scientist working within the central offices of the Ministry of Health and responsible for the NRL and should act as a focal point for malaria QA and be responsible for expanding the service to include a number of other diseases when applicable.

2.3.2 Responsibilities

Programme managers will need to demonstrate that:

- there are potential benefits of high quality laboratory services to the improvement of case management of malaria;
- they can plan, implement and supervise programmes that are feasible; sustainable and compatible with the needs of the country; and
- they can ensure that programmes are adequately and sustainably funded.

This will require:

- a clear definition of the role and importance of the laboratory within the planning and management of malaria control activities;
- the recognition of the important role that laboratory staff play in malaria control;
- a commitment to improving the competency and performance at all levels of the QA programme, based on the continual training and assessment of staff at all levels of the laboratory services, including the establishment of a national core group of accredited expert microscopists;
- ensuring feedback and continual dialogue between all levels of the laboratory network;
- effective use of the programme to follow up poor performance and improve it through support, encouragement and continuing education;
- a sense of ownership and responsibility of all staff;
- benchmarking to allow the comparison of all laboratories in the network;
- a cost-effective plan of action with both a realistic time frame and budgets commensurate with the activities to be carried out; and
- strengthening the integration of malaria QA with other institutions conducting QA within the country, such as national public health laboratories, university departments and NGOs.
2.4 Essential functional elements of the programme

The essential functional elements of each QA programme are:

- a realistic plan of action developed according to a situation analysis;
- development of a budget commensurate with the plan of action, including adequate funding at all levels of the QA programme;
- a network of laboratories to carry out the functions of the programme, including a national reference centre/laboratory for the production of standard operating procedures, training and reference materials such as slide banks;
- a selection, training/retraining and assessment programme that ensures a competent workforce of laboratory staff and trainers;
- a support network that ensures that the performance of microscopists is maintained at the required standards, including:
  - a quality control system based on a benchmarked cross-checking protocol and regular supervisory visits\(^4\), particularly at the initiation of the programme and when poorly performing laboratories are identified;
  - an effective logistics system to supply and maintain the essential reagents and equipment;
  - regular quality control of the routine laboratory operations by laboratory staff;
  - a system to maintain equipment, particularly microscopes, in working order;
- a monitoring system to ensure that the standards are maintained and a culture of quality is developed throughout the QA programme.

2.5 Tasks to be carried out by microscopists

2.5.1 Malaria diagnosis

It is essential that staff at all levels of the QA programme are provided with job descriptions that clearly indicate their responsibilities and define the tasks that they have to carry out. The minimum competencies for a basic malaria microscopist are given in Table 1.

2.5.2 Quality assurance

QA will not be effective unless all involved personnel understand its principles and practices. Training in QA can either be a separate process or incorporated into training/assessment courses for malaria microscopy. The major topics for training basic malaria microscopists in QA are given in Table 2.

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

\(^4\) See Chapter 8
### Table 1. Minimum competencies for a basic malaria microscopist

<table>
<thead>
<tr>
<th>Competency requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood film preparation</strong></td>
</tr>
<tr>
<td>Blood collection</td>
</tr>
<tr>
<td>Cleaning of microscopy slides</td>
</tr>
<tr>
<td>Slides storage</td>
</tr>
<tr>
<td>Preparation of thick and thin films</td>
</tr>
<tr>
<td><strong>Staining</strong></td>
</tr>
<tr>
<td>Correct dilution, quality testing and use of prepared stock of Giemsa stains</td>
</tr>
<tr>
<td>Correct preparation, quality testing and use of Field or Jaswant Singh Battacharya JSB stain</td>
</tr>
<tr>
<td><em>(if used)</em></td>
</tr>
<tr>
<td><strong>Microscope</strong></td>
</tr>
<tr>
<td>Basic cleaning/maintenance</td>
</tr>
<tr>
<td>Correctly set up a microscope (correct illumination)</td>
</tr>
<tr>
<td>Correctly use a microscope</td>
</tr>
<tr>
<td><strong>Slide Reading</strong></td>
</tr>
<tr>
<td>Accurately identify asexual stages</td>
</tr>
<tr>
<td>Accurately differentiate between Pf and non-Pf</td>
</tr>
<tr>
<td>Identify all the species present in the local region</td>
</tr>
<tr>
<td>Identify gametocytes</td>
</tr>
<tr>
<td><strong>Quantification</strong></td>
</tr>
<tr>
<td>Perform a basic differential count on the thick film – neutrophils, lymphocytes, eosinophils</td>
</tr>
<tr>
<td>Identify other major local blood parasites</td>
</tr>
<tr>
<td><strong>Data</strong></td>
</tr>
<tr>
<td>Recording of results in a laboratory register</td>
</tr>
<tr>
<td>Regular collation of data</td>
</tr>
<tr>
<td><strong>Other</strong></td>
</tr>
<tr>
<td>Basic inventory control and stock management</td>
</tr>
<tr>
<td>Training in basic quality control</td>
</tr>
<tr>
<td>Training in blood safety</td>
</tr>
<tr>
<td>Biosafety/waste management</td>
</tr>
<tr>
<td>Use of referral system</td>
</tr>
</tbody>
</table>

#### 2.6 The role of clinical staff in QA

The rational requesting of tests by clinical staff is also an important issue that impacts on the operation of laboratory services. This requires that the clinicians undertake at least a basic history and targeted physical examination of the patient and have the necessary equipment to do so, as well as being able to act appropriately in cases of fever, where no parasites are demonstrated (i.e. cases that are not malaria). Misuse of laboratory services by medical staff wastes scarce resources and may lead to poor patient care.

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5 Giemsa stain is the recommended “gold standard” although a limited number of countries also use JSB or Field stains. See also Chapter 5. Section 5.5.
The time taken for the laboratory to provide accurate results of a blood slide examination to the clinician is crucial to effective treatment and for patients to have confidence in and satisfaction with the health system. In malaria, providing results within 30–60 minutes is considered satisfactory. This goal requires not only an improvement in laboratory services but also that the clinicians and laboratory personnel work as a team with mutual benefit and respect.

Unfortunately, this mutual respect and confidence is not always evident. Clinicians sometimes misuse malaria microscopic diagnosis by proceeding with treatment before the laboratory results are returned, or give antimalarial therapy in cases where no malaria parasites are demonstrated, and therefore not indicated. This may be due to a lack of confidence in the laboratory results. Improving the quality of the laboratory can improve confidence in the results of the blood film analysis by both clinical staff and patients.

Various practices can be put in place to improve confidence of clinicians in microscopy results, including:

- Advocacy aimed at health care providers and patients which emphasizes the importance of malaria slide examination for correct diagnosis;
- The provision of training and support literature for clinicians regarding the clinical importance of malaria microscopy examination and guidelines for requesting slides in situations with differing malaria prevalence;
- The prominent display in the test centres of “Training Certificates” awarded to competent resident microscopists;
- The provision of personal log books certifying competence of each individual microscopist; and
- Maintaining regular supervision and cross-checking of routinely taken slides to confirm a continuing high standard of performance.

### Table 2. Minimal training requirements in QA for basic malaria microscopists

<table>
<thead>
<tr>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consequences of deficient malaria laboratory services</td>
</tr>
<tr>
<td>Basic principles of laboratory quality assurance</td>
</tr>
<tr>
<td>Sources of errors in malaria microscopy</td>
</tr>
<tr>
<td>Essential elements of internal quality control</td>
</tr>
<tr>
<td>Principles and practices for supervisory visits</td>
</tr>
<tr>
<td>Selection and dispatch of slides for blinded cross-checking</td>
</tr>
<tr>
<td>Procedure for cross-checking malaria blood slides</td>
</tr>
<tr>
<td>Quality improvement (corrective actions) in malaria microscopy</td>
</tr>
<tr>
<td>Effect on quality of equipment, reagents, stock control, workload, registration and reporting</td>
</tr>
<tr>
<td>Blood safety</td>
</tr>
</tbody>
</table>
Chapter 3

The Plan of Action

It is the responsibility of the national QA manager/coordinator to organize the development of the plan of action for programme implementation. Implementation should be step-wise and progressive, with intermediate objectives required to reach the ultimate goal of a fully functioning QA system.
3.1 Goals and objectives

The long-term aim of all countries should be to develop a fully functional national QA system with the benchmarking and competency accreditation of all microscopists.

In order to achieve a fully functioning QA system, all QA programmes will need to:

- improve the overall competency and performance of microscopists at all levels of the laboratory services;
- sustain the highest level of accuracy (both in sensitivity and specificity) in confirming the presence of malaria parasites;
- monitor systematically laboratory procedures, reagents and equipment, and the results of laboratory diagnosis; and
- establish a clear hierarchical reporting system for results of QA.

The time required to reach these goals will vary between countries since it depends on resources available, the structure of the health system, the laboratory network available and the incidence of disease.

A model for the progressive implementation of QA is given in Figure 2.

Figure 2. Progressive implantation of QA in different contexts

- Establish the infrastructure with National Reference Centre, a laboratory network and a national slide bank. Provide equipment and supply lines for reagents and consummables.
- Selection / training of microscopists

- Basic QC to identify the poorest performing laboratories
- Supervisory visits and/or validation by cross-checking of routine slides
- Competency accreditation of national and regional microscopists

- Benchmarking. Comprehensive cross-checking of slides with continuous improvement of all laboratories (poor, satisfactory, top performing)
- Establishing minimum performance standards based on actual laboratory performance
- Competency accreditation of national and provincial expert microscopists

- Laboratory accreditation based on internationally accepted best practice performance standards
Chapter 3: The Plan Of Action

Thus, the intermediate objectives of each national QA programme will have to be adapted to the varied contexts in which they are being developed. For example:

- In countries lacking the infrastructure and adequately trained staff, evaluation of existing laboratory services might not be feasible in which case priority should be given to the training of microscopists and building up the necessary infrastructure for them to carry out their tasks effectively.

- In countries with limited infrastructures and poorly performing laboratory services, the intermediate objectives should be to identify and improve poor performing laboratories and personnel, and competency accreditation of national and regional microscopists.

- In countries that already have a functioning QA system with trained personnel and some form of infrastructure, the objective should be to aim for the “bench-marking” of all laboratories to the highest standard, establishing minimum performance standards based on actual laboratory performance and competency accreditation of national and regional microscopists.

3.2 Essential elements

Continuous interaction between national laboratory experts, clinicians and epidemiologists will be essential in developing a plan of action as well as its implementation and the monitoring of all activities for improving the QA of malaria microscopy.

The essential elements of the plan of action are:

- its context within the goals and priorities of the National Laboratory Services and the NMCP;
- an analysis to assess the current situation;
- the specific objectives and goals of the QA programme;
- the expected outcomes of the programme;
- a list of constraints which might affect the achievement of these objectives; and goals;
- a list of the activities to be carried out;
- a timetable of activities;
- a detailed and realistic budget; and
- a list of indicators for measuring progress and outcomes of the QA programme with appropriate reporting forms.

3.3 Pre-implementation assessment

The first step of the plan of action should be a situation analysis to determine the current status of QA in the country.

This analysis should result in a reasonably accurate estimate of the resources required to ensure that QA can be implemented and be sustainable. The following is a checklist of
factors that will affect the effective implementation of the QA system:
- the current objectives of the malaria control programme and the role of parasitological confirmation of malaria;
- current organization of the laboratory services for malaria diagnosis;
- the status and/or feasibility of integration with national laboratory services (this will depend on the specific objectives of the malaria control programme);
- the role and importance of the private sector and NGOs in malaria diagnosis and treatment;
- the existence and capabilities of a National Reference Laboratory;
- capabilities of the existing infrastructure and staff for training and assessing the competence and performance of the laboratory services;
- current availability of reagents and equipment;
- capabilities of existing logistic systems to ensure provision the necessary reagents and equipment and maintain the latter in working order;
- availability and use of guidelines and standard operating procedures for all activities related to ensuring a high quality of malaria microscopy;
- reporting mechanisms;
- current organization, status and performance of existing quality assurance; and
- current levels and origins of financial support for strengthening malaria diagnostic services.

Key issues to be considered in the situation analysis:

- Are the laboratory environments at all levels appropriate for the work to be performed?
- Are the staff numbers adequate for the workload?
- Are the operating procedures up-to-date and followed by all staff?
- Are all staff adequately trained in the tasks that they have to 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 the supplies of reagents and equipment adequate?
- Is there adequate budgetary provision for envisaged tasks to be carried out?

The recommended steps for this situation analysis are given in Table 3.
### Table 3. Recommended steps for pre-implementation situation analysis

<table>
<thead>
<tr>
<th>Tasks</th>
<th>Key Issues</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Make a chart of the laboratory network showing relationships and functions of the different levels.</td>
<td>The network should be supervised by a national reference laboratory. Laboratories at the intermediate levels should support peripheral laboratories.</td>
<td>Where a formal network has not yet been established a provincial or regional laboratory may support QA in peripheral laboratories as an interim measure.</td>
</tr>
<tr>
<td>2. Make an inventory of available resources (include staff, microscopes, equipment and budget).</td>
<td>Microscopists should have received appropriate training in malaria microscopy. This will require an effective training (assessment) programme designed for the specific 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 x7 or x10 eyepiece and x100 oil immersion objective in good working order (plus x40 objective for non-malarial work) and the capability of microscope maintenance. The laboratory should have all the facilities to allow high quality malaria microscopy examinations to be carried out. There should be regular communication between the laboratory and both the clinical staff requesting the diagnosis and the NMCP. Laboratories should have appropriate administrative support.</td>
<td>Microscope performance is critical to providing a quality diagnostic service. Replacement of defective microscopes may not always be necessary if an effective servicing is available. Electrical binocular microscopes are mandatory. Microscopy with direct light (sunlight) is not acceptable as there is sub-optimal resolution at low light intensities. If possible the type of microscope in use should be standardized throughout the laboratory services.</td>
</tr>
<tr>
<td>3. Collect data on the current workload and assess adequacy of resources with respect to workload.</td>
<td>Staffing levels should be sufficient to provide an effective and sustainable service (see section 3.4. Workload).</td>
<td>Excessive workloads are a major contributor to poor performance. Laboratories with abnormally high or low workloads should be identified.</td>
</tr>
</tbody>
</table>
3.4 Workload

Excessive workloads are a major factor contributing to poor performance. Sensitivity is directly related to the time available to examine blood films and therefore decreases when the number of slides exceeds the workload capacity of the microscopist. Even highly competent microscopists cannot perform at their best if they do not have the time available to correctly examine malaria slides. This problem is compounded where microscopists have responsibilities for diagnosing other diseases.

The longstanding WHO recommendation made during the eradication era that a person can satisfactory read 60–75 slides/day is now considered to be unrealistic as a standard since the functions and role of malaria microscopists in malaria control are different today. The time taken to confirm the absence of parasitaemia, as is the case in most cases of febrile illness likely to be selected for microscopy-based diagnosis, precludes such a rapid
turnover of slides. In addition, accurate quantitation of parasites, which is important in many situations where microscopy is used, also takes considerable time. It is difficult, therefore, to recommend a number of slides that represents a reasonable workload for all situations as parasite prevalence varies, and the workload capacity of an individual microscopist is dependent on many factors.

The time required to read an individual malaria blood film depends on:

- the quality of the microscope and the laboratory organization;
- the skill of the microscopist;
- the slide positivity rate (SPR);
- the parasite density;
- the time required to reading positive and negative slides as strongly positive thick films can be examined considerably more quickly than weak positive and negative films, therefore, slide reading capacity rises with increasing slide positivity rates and at higher average parasite densities; and
- the balance of accuracy of quantitation versus the efficiency required to guide clinical decision-making.

Another significant factor is the additional time required for species identification in regions where this is clinically important. This will depend on whether this is must be performed on the thick or thin film. Species identification from thin films at low parasite density is extremely time-consuming.

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

**Table 4. Times used to calculate the minimum total time to examine a thick blood film for malaria parasites**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Minimum time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locating and placing the slide on the microscope stage</td>
<td>5 seconds</td>
</tr>
<tr>
<td>Adding oil and focusing the x100 objective</td>
<td>5 seconds</td>
</tr>
<tr>
<td>Microscopic examination of a high density positive thick film to determine +ve or -ve</td>
<td>10 seconds</td>
</tr>
<tr>
<td>Microscopic examination of a low density positive thick film to determine +ve or -ve</td>
<td>~2–6 minutes</td>
</tr>
<tr>
<td>Microscopic examination of a negative thick film</td>
<td>6 minutes</td>
</tr>
<tr>
<td>Counting the number of parasites/200 WBC in +ve film</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Recording the result in the register</td>
<td>5 seconds</td>
</tr>
</tbody>
</table>

The actual time required for each step will be likely to vary between programmes. However the timings given above approximate to the reading capacity of a trained basic malaria microscopist. It must be noted that a very rapid reading of a slide with high parasite density will give an indication of the presence of malaria parasites, but give no indication of the presence of a mixed infection.
Furthermore, the number of slides that can be read is also dependent upon whether the individual microscopist:

- only performs microscopy, or has other duties apart from malaria diagnosis;
- only stains and examines the films, or;
- performs all the functions required to obtain a malaria microscopic diagnosis (including the collection of blood from the patient, the preparation and staining of the blood slide and its microscopic examination); and
- has other duties apart from malaria diagnosis.

An acceptable workload will, therefore, vary depending on the context.

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

- Extended hours of continuous reading result in fatigue that can significantly lower the accuracy of reading.
- In many clinics and hospitals the majority of patients arrive in the morning and therefore microscopy is concentrated into a peak period rather than being distributed across the whole working day.

The following table is calculated by estimating that it takes 30 seconds to read a strongly positive malaria slide and 6 minutes to read both a weakly positive and a negative slide (using estimates from Table 4), assuming roughly half the infections are of high parasite density and half are low.

<table>
<thead>
<tr>
<th>Slide Positivity Rate</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slides/hour (no quantitation)</td>
<td>10</td>
<td>10.5</td>
<td>11.1</td>
<td>11.7</td>
<td>12.3</td>
</tr>
<tr>
<td>Slides/4 hours (no quantitation)</td>
<td>40</td>
<td>42</td>
<td>44.4</td>
<td>46.8</td>
<td>49.2</td>
</tr>
<tr>
<td>Slides/6 hours (no quantitation)</td>
<td>60</td>
<td>63</td>
<td>66.6</td>
<td>70.2</td>
<td>73.8</td>
</tr>
<tr>
<td>Slides/hour (quantitation)</td>
<td>9</td>
<td>8.5</td>
<td>8.1</td>
<td>7.6</td>
<td>7.3</td>
</tr>
<tr>
<td>Slides/4 hours (quantitation)</td>
<td>36</td>
<td>34</td>
<td>32.4</td>
<td>30.4</td>
<td>29.2</td>
</tr>
<tr>
<td>Slides/6 hours (quantitation)</td>
<td>54</td>
<td>51</td>
<td>48.6</td>
<td>45.6</td>
<td>43.8</td>
</tr>
</tbody>
</table>

If the microscopist also collects and/or stains the slides, the daily slide output will be significantly reduced. For example, collection and staining might requires 6 minutes, resulting in the time required to read strongly-positive slides increasing to 6.5 minutes, and to 12 minutes for a weak or negative slide, thus reducing the average slide output significantly.
3.5 Costing of QA programmes

It is clear that costs of implementing a national QA programme will vary greatly between countries due to a variety of factors, such as the:

- specific goals of each country, i.e. malaria control or elimination
- percentage of the population at risk of malaria
- status and effectiveness of present system
- each country’s implementation capabilities, including the number of laboratories involved in the programme.

Preliminary studies suggest that the implementation costs will be relatively low in those countries where there are existing infrastructures and trained staff for QA. For countries requiring the scaling up of quality assurance, short-term costs will be higher because of the need for procurement and refurbishment of equipment and for increased human resources needed to for the training and retraining of microscopists and supervisors.

The system for producing programme budgets may vary between countries but, whichever accounting system is used, budgets should be realistic and commensurate with the activities to be carried out. The essential components for costing the plan of action for QA implementation are as follows:

3.5.1 Initial costs

(i) Central and Intermediate Levels
   - Establishment of the post of national malarial QA coordinator
   - National meeting to develop QA strategy
   - Development of a national slide bank
   - Development of National Reference Group or Laboratory
   - Pre-implementation situation analysis
   - Training of expert microscopists for supervisory role
   - Purchase of slides and equipment

(ii) Peripheral Level
   - Training of basic microscopists
   - Laboratory improvement
   - Laboratory equipment and supplies
   - Communication

3.5.2 Recurrent costs

(i) 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
Supervisory visits
Slide shipment and associated administrative costs
Annual meeting for programme review

(i) Peripheral Level
Laboratory supervision
Annual replenishment of supplies and equipment
Laboratory and equipment maintenance
Administration and communication
Chapter 4

Supplies and Equipment

High quality microscopic diagnosis of malaria requires an effective logistics system to provide standardized reagents, supplies and equipment which are maintained working order.
4.1 Standardized lists

The ability to perform high quality work depends directly on the quality of the equipment and reagents available. There is great variation in the type and standards of equipment and reagents used by countries with the result that many laboratories need guidance. Major problems also exist in operating an effective logistics system that can maintain adequate supplies and the equipment in working order.

It is recommended that the NMCP should develop and endorse guidelines on the equipment and reagents needed to perform malaria microscopy. This should also include:

- a list of the minimum standards for equipment and supplies;
- specific recommendations for the selection of microscopes; and
- guidelines for assessing microscope used in the field to ensure they are operating correctly.

These guidelines should also take into consideration the different contexts in which national programmes are operating.

All equipment and supplies should match the national or international recognized standards. When this is not immediately possible, standardization should take place as soon as possible. The standardization of microscopes (electric binocular microscopes are mandatory) is essential as this simplifies both maintenance and the acquisition and supply of spare parts.

A model list of supplies and equipment for the establishment of a peripheral laboratory with a workload of 1000 slides during a 3-month period is given in Annex 1.

4.2 Establishment of a supply chain

It is essential that an effective supply chain be established to foresee and provide all the equipment and supplies that are needed to sustain an uninterrupted flow of reliable malaria diagnosis. To facilitate this, standard establishment and replenishment lists of equipment should be created. Replenishment should be on an “as-and-when” required basis, but if rapid replenishment of consumable items cannot be assured, buffer stocks equal to at least six month’s operational requirements should be held at all levels.

4.3 Microscopes

A reliable and well maintained microscope is an essential requirement for accurate malaria microscopy. A binocular microscope with an x7 or x10 eyepiece and an oil immersion lens (x100) with a built-in electrical light source is the “gold standard”. In the absence of a binocular microscope, a monocular version may be used but this leads quickly to eye fatigue with a subsequent reduction in the performance of the microscopist. The use of blue filters to change the light from ordinarily electric bulbs to a more natural white light is also recommended. Immersion oil with a refractive index of 1.5 should be of high quality and used according to the manufacturer’s recommendations.
4.4 Microscope slides

Only high quality microscope slides, free of surface abrasions and purchased from a reputable dealer, should be used for malaria microscopy. They 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 which confuse malaria diagnosis and will avoid the detachment and washing away of thick blood films during the staining process.

It is not generally recommended that slides should be reused although, in some circumstances, when unused slides are unavailable, this may be necessary.

4.5 Stains

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

The alcohol based Giemsa stain is the “gold standard”. It is the most commonly used stain and the best for routine diagnosis due to its applicability to both thick and thin blood films, its stability during storage and its constant and reproducible staining quality over a range of temperatures. Although it is relatively expensive, it is the stain of choice for peripheral laboratories.

In view of its critical importance in producing fine quality staining, Giemsa stain powder should be bought from a reputable supplier and Giemsa stain stock solutions should be made up by the QA programme in quality controlled batches and distributed in-house to the end-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 exist and should be available to all malaria diagnostic laboratories as pH paper is not satisfactory for the pH measurement of water and buffers, both because small differences in pH (such as between pH 7.0 and pH 7.2) can make significant differences in stain quality, and pH paper is unable to measure sufficiently small pH increments.

Field and JSB stains are the most widely used aqueous-based stains. Field stain has the advantage that the film is stained in one minute. It is useful in laboratories where the workload is low and an excellent stain when used properly but variable results may occur during routine use, thereby reducing its widespread application. JSB stain has been used extensively throughout the Indian subcontinent. However, it requires subtle handling, an art that many have failed to acquire. It has not been used outside India for many years.
A national reference (core) group of expert microscopists, accredited to internationally recognized standards for malaria microscopy, is an essential part of all malaria QA programmes.
The national reference (core) group of microscopists needs to be proficient in all of the tasks required to prepare blood films, accurately diagnose and report the results from the examination of malaria blood slides. This group of experts may include trained medical or laboratory personnel, or on occasion other health workers trained in malaria microscopy.

The selection of these microscopists should be based on an objective evaluation made during assessment courses specially designed for potential high level malaria microscopists. Those who reach the required levels of competency, based on practical and sometimes written tests, should be accredited according to the competency they attain.

The assessment detailed in this chapter is aimed at senior microscopists who are expected to act as reference clinical microscopists for slide cross-checking (validation) and training within the national malaria microscopy programme. It is assumed that these personnel have a high level of competency before entering the assessment. The assessment should be conducted under conditions similar to those of a good, operational reference malaria laboratory. Accreditation of lower level microscopists within the national malaria microscopy programme should be conducted through a modified protocol appropriate to the local needs (e.g. with less emphasis on accuracy of parasite density determination), and may need to be preceded by an appropriate re-training programme. Accreditation of expert microscopists for clinical trials usually requires a more stringent assessment geared to the specific requirements of the trial.

Competency assessments should be combined with some form of retraining activity, preferably following a pre-test that identifies specific areas needing revision. The proportion of time devoted to retraining, and so the length of the course, will depend on prior expertise of the participants. As leading microscopists, the participants will also need to be competent teachers of malaria microscopy, and to be effective managers and supervisors within the national programme, consideration should be given to addressing these needs through additional training.

5.1 Aims of accreditation

Accreditation of the competency of each microscopist has the potential to significantly improve the QA of malaria diagnosis, raise self-esteem and enhance career development, if linked to a defined career structure with pay enhancement.

It is strongly recommended that national programmes:
- give formal recognition to the skill levels of individual microscopists;
- develop a training programme that links training with an accreditation system that accredits appropriately the competence of microscopists for working at each level of the QA laboratory network;
- monitor this competency continuously; and
- provide a career path for accredited microscopists.

It is recommended that accreditation should last for not more than three years. Earlier reassessment should be available for those who are not performing well. Accreditation of individual microscopists is separate from both other forms of QA and validation (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 accreditation level. It is recommended that microscopists have an individual Training/Competency Logbook where relevant certificates and qualifications are recorded. This should be held and kept up to date by the individual microscopist with a copy kept by the laboratory supervisor.

5.2 Principles of assessment

Competency assessment should be based on the detection of malaria parasites, species identification and on accuracy of parasite quantitation.

Each participant must be informed, in a completely transparent manner, of their performance within the group and their resultant competency grading. The examination results need to be openly discussed, and utilized constructively rather than punitively.

Assessments should set performance targets that are realistic, achievable and sustainable, and should include, at a minimum, grading of microscopy competence as parasite identification (presence, and species identification) and parasite counting (quantitation). Formal assessment and grading of blood film preparation and staining, microscope use and maintenance, blood safety and malaria knowledge may also be included, but graded separately from microscopy competence.

5.3 Planning accreditation courses

It is recommended that the programme of microscopist accreditation should start in each country with participants who have the potential to work at National (central) level, preferably including the national malaria reference laboratory. Assessment of regional/intermediate staff can follow when sufficient national staff have been certified as competent for the tasks that they have to perform within the QA programme.

It is essential that participants in these national reference (core) group accreditation assessments have already been fully trained. They should be experienced microscopists.

The course should always attempt to raise competence, as well as assess it. However, the time devoted to assessment must be sufficient to ensure that microscopists with poor competency are not accred ted as sufficiently competent, and that expert microscopists are appropriately recognized.

5.3.1 The aim

The primary aim of these courses is to objectively and formally assess the competency of malaria microscopists.

An important secondary consideration is to provide refresher training, based on standardized instruction and revision.
5.3.2 The trainer/facilitator

One-to-one interaction between the trainer/facilitator and the participants is essential. The number of participants should, therefore, be strictly limited, e.g. a maximum of 12 in a group. The trainer/facilitator must be highly experienced and have proven competency. They must also have the ability to create a relaxed and respectful atmosphere. Each WHO region will need to develop a pool of such trainers/facilitators.

5.3.3 Syllabus and timetable

The course should last a minimum of five days to allow sufficient interaction and the assessment and enhancement of the required skills. It is often difficult for a clinical microscopist to be away from their job for more than five days to attend a course. The curriculum must address the requirement for rigorous assessment of competence while addressing the capacity of an active diagnostic programme to operate with some laboratory staff absent. At a minimum, the assessment must grade:

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

Refresher training should include, at a minimum:

- preparation and staining of blood films;
- biosafety;
- correct use and maintenance of the microscope; and
- proper QA and QC.

The programme should include a theory (Annex 3) and practical pre-test at the commencement of the course (with immediate feedback) and a blinded end-of-course practical assessment on which accreditation should be based. A model syllabus and timetable for an assessment course for senior microscopists is given in Table 6. Review and revision sessions should include the identification of artefacts, including films containing bacteria and fungi, and common staining errors.

Participants should be given a copy of the course curriculum and relevant SOPs at least one month prior to the course, to allow time for preparation and revision. Where possible, other revision material such as CD ROMs of blood films, or slide sets, should also be available.
Table 6: Model training course for competency assessment of national / intermediate-level malaria microscopists

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Registration</th>
<th>Opening &amp; Orientation</th>
<th>Pre-Workshop Theory Test</th>
<th>Break</th>
<th>Theory Test Feedback</th>
<th>Microscope Use &amp; Blood Elements</th>
<th>Lunch</th>
<th>Pre-Workshop Practical Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td>Review of Practice Slides</td>
<td>Malaria Overview &amp; Parasite Quantitation</td>
<td>Break</td>
<td>General Revision (Species ID)</td>
<td>Group Slide Review</td>
<td>Lunch</td>
<td>Test Slide Examination (Slide set 1)</td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>Review of Test Slides</td>
<td>Counting Techniques</td>
<td>Test Slide Examination</td>
<td>Break</td>
<td>Test Slide Examination (Slide set 1)</td>
<td>Lunch</td>
<td>Test Slide Examination (Slide set 1)</td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>QA in Malaria lab Diagnosis &amp; SOP/doc updates</td>
<td>Break</td>
<td>CD &amp; Internet Revision</td>
<td>Group review of counting</td>
<td>Lunch</td>
<td>Test Slide Examination (Slide set 2: Quantitation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td>Review of Test Slides</td>
<td>Revision Malaria RDTs optional</td>
<td>Break</td>
<td>Course Evaluation and Interviews</td>
<td>Final Interviews (if required)</td>
<td>Lunch</td>
<td>Graduation and Close</td>
<td></td>
</tr>
</tbody>
</table>

6 The assessment detailed here is intended for senior technicians who are expected to act as reference clinical microscopists for slide cross-checking and training. Accreditation of lower level technicians and accreditation for expert technicians in clinical trials should be conducted through modified protocols adjusted for the needs of these situations.
5.3.4 Requirements for equipment and supplies

Prior to commencement of the workshop, it is vital that the staff of the institute hosting the course ensures that sufficient numbers of good quality and well-maintained microscopes, tally counters and calculators are provided for the participants. A multi-headed microscope is particularly useful and should be provided if possible. Consumables such as immersion oil and lens tissue are also required, as is spare equipment, including back-up microscopes and spare light bulbs for the microscopes (see Annex 2 for a model list of essential equipment).

5.3.5 Blood slides

Parasites in the majority of slides used for training and assessment must reflect the locally prevalent species, taking into consideration that the species incidence and parasite density may vary seasonally in many countries and areas. Blood slides must be of high-quality and carefully validated before use. All blood slides should be checked for species identification by polymerase chain reaction (PCR). Parasite densities should be validated by multiple expert microscopists, who participate in external quality assurance programmes. Additionally, the assessment procedure and the course facilitator must be sufficiently competent and flexible to account for errors in previously validated slides.

The recommended WHO competency assessment panel (Box 1) consists of 2 sets of slides:
- one set of 40 slides designed to assess a microscopists’ ability to detect the presence or absence of malaria parasites, and identify the species;
- one set of 15 malaria positive slides (P. falciparum only) designed to assess a microscopists’ ability to accurately estimate parasite density (count parasites).

Each slide should include a thick and thin blood film. At high parasite densities (>2 parasite/white cell), course participants should be allowed to count and record parasite density against either white cells (thick film) or red cells (thin film), according to local policy. High-density slides must be validated against both standards separately.

Wherever possible, the examination of slides of unusual presentation, including drug-affected parasites, poor staining, very high and low parasite densities, combinations of mixed infections, and those with 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 training component of the course. These slides should currently not be included in the final assessment, as standardization between courses and countries is important.
Chapter 5: Establishing A National Reference Group of Expert Microscopists

Box 1 Model Minimum Slide Sets for Accreditation of Trained Laboratory Staff in Malaria Microscopy at National Reference (Core) Group Level.

Slide set 1 (40 slides): Assessment of presence/absence of parasites, and species identification
- 20 negative slides:
  - 20 'clean' negatives
- 20 positive slides of low density (80-200 parasites/µL):
  - 10 *Plasmodium falciparum* slides
  - 4 mixed (2) species slides (Include *P. falciparum*. Each species >40 parasites/µL, co-infecting species according to local prevalence)
  - 6 of *Plasmodium malariae*, *Plasmodium vivax*, and/or *Plasmodium ovale* slides (include at least 1 of each species, ratio according to local prevalence)

Time limit: 10 minutes per slide

Slide set 2 (15 positive slides): Assessment of quantitation
- 3-5 *P. falciparum* (200-500 parasites/µL)
- 9-10 *P. falciparum* (500-2000)
- 2 *P. falciparum* >100 000 parasites/µL

Time limit: 10 minutes per slide

5.3.6 Standard operating procedures

All malaria diagnostic programmes must have SOPs covering basic microscopy maintenance, blood film preparation and staining. Any SOPs to be used in the course should be provided to participants, together with the curriculum, at least one month prior to the course. An example of a SOP for slide preparation is given in Annex 8 and in the WHO *Basic Malaria Microscopy Manual Part One. Learners’ Guide*.

Box 2. Achieving Consistent Parasite Quantitation

When assessing a thick film, it is important that a wide area of the film be scanned to reduce the effect of inconsistencies in film thickness on detection and parasite density determination of parasites. This is achieved by moving a wide area of the thick film across the objective according to a systematic pattern, while the required number of fields are read. Different microscopists have preferred techniques to achieve this. The important principle is that the area viewed (e.g. 100 fields) is not restricted to a small section of the thick film. This should be conveyed clearly during assessment courses.

5.4. Basic elements of assessment

The competency of all participants should be assessed both before and at the end of each course. The final assessment should be more extensive, and act as the basis for competency accreditation. The course should include revision of the basic elements of malaria microscopy, and include opportunity for one-to-one review of difficult slides and of reading errors in the pre-test.

5.4.1 Conditions for assessment

A relaxed atmosphere is important, and participants should have the opportunity to familiarize themselves with the environment and equipment prior to the assessment. Strict examination conditions should be maintained to ensure quietness and confidentiality. It is recommended that the assessment be spread over three days, as shown in the model timetable given in Table 6.

High-quality binocular microscopes with electric light should be provided and the participants allowed the appropriate time for each of the slides used for assessment (Box 1). Each assessment slide should be coded and provided in a random order. The code should be changed each day and the assessment spread over three days (if following the recommended WHO standard).

Written and pictorial reference materials should be allowed during the assessment because it aims to evaluate the participant’s competence in a normal working environment rather than the participant’s recall. Each candidate should therefore have access to materials such as WHO’s Bench Aids8 for the diagnosis of malaria infections and be free to bring in their own references. However, any additional documentation must be checked for accuracy by the trainer/facilitator.

5.4.2 Pre-course assessment

The pre-course assessment (pre-test) of each participant should be based on a written theory test (see Annex 9 for a model examination paper) and a pre-test on the participant’s competency in examining a similar but smaller panel than that used for the final assessment. This will allow a comparison to be made between the results of the pre-test and the final assessment.

5.4.3 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 use high-quality and well validated blood slides, and not rely on the facilitator’s judgment.

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8 Bench Aids for the diagnosis of malaria infections are aimed at malaria microscopists and designed in the form of 12 plasticized plates for the day-to-day use in the laboratory. They can also be used for teaching purposes.
The recommended slide panel for the final course assessment is given in Box 1. Each slide should include a thick film and a thin film. This panel is designed for assessment of the WHO recommended competency accreditation standards.

Some assessments may extend this panel for specific reasons, such as ensuring a very low false-positive rate for certain drug trials.

### 5.5. Minimum grades for expert accreditation

The slide panel currently recommended by WHO is shown in Box 1. This panel is based on experience with assessments in Asia and Africa, and reviewed by WHO consultations in 2006 and 2008. The slide set panel and grading system are designed to ensure chance (variation in parasite distribution in a film) is highly unlikely to result in incorrect grading of a microscopist under assessment.

<table>
<thead>
<tr>
<th>Accreditation Level</th>
<th>Detection of parasitaemia</th>
<th>Species Identification</th>
<th>Parasite Quantitation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slide set 1</td>
<td></td>
<td>Slide set 2</td>
</tr>
<tr>
<td></td>
<td>(40 slides)</td>
<td></td>
<td>(15 slides)</td>
</tr>
<tr>
<td>Level 1 (Expert)</td>
<td>³ 90%</td>
<td>³ 90%</td>
<td>³ 50%</td>
</tr>
<tr>
<td>Level 2</td>
<td>80% - &lt;90%</td>
<td>80% - &lt;90%</td>
<td>40% - &lt;50%</td>
</tr>
<tr>
<td>Level 3</td>
<td>70% - &lt;80%</td>
<td>70% - &lt;80%</td>
<td>30% - &lt;40%</td>
</tr>
<tr>
<td>Level 4</td>
<td>&lt;70%</td>
<td>&lt;70%</td>
<td>&lt;30%</td>
</tr>
</tbody>
</table>

Parasite detection, identification and quantitation criteria must all be met for accreditation. Certificates should include the grade number (1–4) to allow comparisons to be made between different countries which may issue the certificates in local languages. Certificates should state the due date for the next assessment (a maximum of three years). There should be provision for a requirement for earlier re-training and accreditation if poor competency is detected through validation and supervision activities of the QA programme.

Accreditation programmes may consider expanding the theory course to include assessment of knowledge through written tests and practical aspects of blood film preparation and microscope maintenance. This will depend in part on programme needs and time available, and will require the course to extend beyond five days.

9 Each slide should contain a thick and thin film. To count parasites, participants should read the thick film at lower parasite densities, but be allowed to count using either film in slides with high parasite density (>2 parasites/WC) in order to reflect normal working conditions. It must be noted that parasite counts on a thin film are commonly higher.

10 Statistical analysis available from WHO
The slide set and accreditation standards used in this chapter are designed for clinical microscopists working as reference microscopists and/or trainers in national programmes, in a context where there is limited time available during which microscopists with important programmatic roles can removed from their normal duties. For specialized roles, such as microscopy in drug or vaccine trials, it is recommended that the slide set be expanded to more thoroughly assess areas in which the results are very sensitive, such as increasing the size of the negative slide set.
Chapter 6

Training of Clinic (Peripheral) Level Microscopists

Well trained and competent microscopists are essential for rapid diagnosis and prompt treatment of malaria at the periphery of the health services.
6.1 Selection of peripheral level microscopists

The appropriate educational requirements for peripheral level microscopists will vary depending on a variety of factors. Experience in many parts of the world has shown that health workers from a wide range of educational backgrounds can be accepted for training as malaria microscopists. However, if the entry level is relatively low, the period of training may need to be extended since it may take longer to train someone with only 8 years of schooling compared to someone with 12 years. On the average, someone with no previous experience of malaria microscopy can be trained to an acceptable level of competency in five weeks.

It is important that peripheral level microscopists:

- are able to read, comprehend and write in the local language;
- can systematically follow a set of written instructions;
- have good hearing and eyesight;
- are sympathetic to the health problems of the community;
- indicate a willingness on the completion of training to work with members of the community, especially those who are sick; and
- in certain circumstances to work for periods in rural areas away from home.

In the past, it was considered that colour-blindness was an exclusion factor for selection for malaria microscopists but there is no data to support this theoretical exclusion. Therefore, it is recommended that trainees should be given eye tests only if difficulties are experienced during training.

Table 8 below summarizes the selection criteria and the recommended length of training for basic microscopists working at the peripheral level.

Table 8. Selection criteria and training requirements for malaria microscopists

<table>
<thead>
<tr>
<th>Selection Criteria</th>
<th>Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persons with no previous experience</td>
<td>Literate, numerate (can read and write at a basic level) If experience difficulties with training, test eyesight</td>
</tr>
<tr>
<td>Laboratory technicians</td>
<td>Previous experience in working in microscopy laboratories</td>
</tr>
</tbody>
</table>

6.2 Training courses for peripheral-level microscopists

6.2.1 WHO training manuals

It is recommended that training courses for basic 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. tutor’s guide which were revised in 2008. The learner’s guide contains all the technical information that will be needed by trainees in
Chapter 6: Training of Clinic (Peripheral) Level Microscopists

this field. The tutor’s guide gives extensive advice for those responsible for organizing, running and evaluating training programmes.

The training manuals are intended to standardize and facilitate the teaching of all the individual tasks involved in malaria microscopy, outlining each step of the diagnostic routine in the sequence that they should be carried out in the laboratory. The style of writing and presentation of the manuals has been kept simple to avoid misunderstanding and to facilitate translation into any language.

The tutor’s and learner’s guides may be used together (and with audiovisual aids) for initial basic training and for in-service training. The Learner’s Guide alone may be also used for refresher training, or by individuals for reference.

Providing trainees with the learner’s guide ensures that:

- all participants are provided with the same set of training materials, allowing uniformity of training, and avoiding errors that can occur when trainees prepare their own notes;
- trainees can directly refer to the notes during training, thereby increasing understanding; and
- after the course, each trainee can take home a set of notes that will be a helpful reference in their daily work.

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

6.2.2 Training objectives

After successfully completing initial training, basic microscopists should have acquired the skills and competence to enable them to:

- understand and describe the importance of malaria as a potentially life-threatening disease;
- describe and demonstrate the special precautions required when handling blood to prevent transmission of blood-borne pathogens;
- describe correctly the common clinical signs and symptoms associated with malaria;
- accurately record patient details and results in the laboratory register;
- prepare acceptable good quality thick and thin blood films with thick and thin films on the same slide;
- correctly stain, with Giemsa (and where appropriate other stains used by the programme), blood films for microscopic examination;
- understand the correct set up and maintenance of a microscope;
- understand the correct procedures used to examine thick and thin blood films;
- correctly identify the components of normal blood to an accuracy of >95%;
- correctly identify malaria ring forms, schizonts and gametocytes to an accuracy of >95%;
- correctly recognize and identify P. falciparum to an accuracy of >95%;
- identify non-P. falciparum malaria species to an accuracy >80%;
- grade or calculated the parasite density to an accuracy of >80%;
record, without errors, the results of their examination on the correct form, and report their findings to those responsible for patient care;
understand the necessity of following the correct procedures, performing basic quality control, reporting findings in a timely manner and understanding stock control; and
have basic training skills to enable them to pass on their knowledge to others.

### BOX 3. Note on Parasite Quantitation in Routine Clinical Practice

#### Reading a thick blood film
When reading a thick film, it is recommended that a minimum of 100 high power fields be read before the film is considered negative (no parasites). If a parasite is detected and quantitation is necessary, parasites should be counted against WBCs. Counting of WBCs and parasites may commence with that field, or from the initial field viewed.

When assessing a thick film, it is important that a wide area of the film is scanned to reduce the effect of inconsistencies in film thickness on detection of parasites and determination of parasite density. This is achieved by moving a wide area of the thick film across the objective according to a systematic pattern, while the required number of fields are read. Different microscopists have preferred techniques to achieve this. The important principle is that the area viewed is not restricted to a small section of the thin film. This should be conveyed clearly during training courses.

#### Counting parasites (quantitation)
Accurate parasite density estimation based on parasites per microlitre or white cell count is necessary when parasite density determination is important for clinical decision-making (for example in severe malaria or where monitoring of treatment efficacy is required) and in clinical trials.

It is recommended in routine practice that parasite quantitation be performed against 200 or 500 WBCs. If, after counting 200 WBC, 100 or more parasites are found, record the results in terms of number of parasites/200 WBC. If less than 100 parasites are found after counting 200 WBCs, parasite quantification should be continued until 500 WBCs are counted. (This gives a probability <5% of chance variation greater than 25% of true parasite density using a x100 oil immersion objective and an eyepiece with a field number of 18). All parasites in the final field are counted even if the count exceeds 500 WBCs. To determine parasite density, the parasite count is adjusted against the true WCC where available. If unavailable, common practice is to assume a WCC of 8000/microL.

Alternatively, parasite density may be determined against a fixed known volume on the thick film (Earl-Perez method) or (in the case of high parasite densities) as a percentage of red cells on the thin film. The method of determining parasite density should be noted.

#### The ‘plus’ system of quantitation
It was previously recommended that a ‘plus’ system for estimating parasite density be used, with ‘+’ used to indicate a low parasite density, and ‘++++’ a very high parasite density, and this system is still widely used in some national programmes. However, inconsistency in the methods used to assess the graduations of density, the area of film scanned, and the step size between graduations, mean that comparisons of parasite density based on the plus system are of very limited clinical value. In most clinical situations, clinical decision making requires only an indication of presence or absence of parasitaemia, and identification of the species present. Therefore, the ‘plus’ system for reporting parasite density is no longer recommended, and should be phased out and replaced by the accurate counting methods described above in situations where parasite density determination is required.

It is recognized that time will be required for clinical staff to become familiar with quantitation reported as parasites/microL and therefore many programmes will need to phase out the ‘plus system’ progressively. When quantitation is taught and assessed in QA programmes, accurate quantitation should be emphasized.
6.2.3 Assessing competency

Evaluation should be an objective assessment of the level of competency that the trainees have achieved in each of the components of the training course. The methods of evaluation and the expected grades of competency for basic peripheral microscopists are detailed below. Evaluation by the trainees of the course, the tutor and the facilitators is also important and will provide feedback that will help in the improvement of future training courses.

Rationale

Assessment of competency at a basic level should acknowledge that many participants will have had only five weeks experience at the time that they take the examination. It is assumed that participants will rapidly build on the basic competencies learnt in the training once they start applying the techniques in regular practice.

Trainees should be monitored continuously throughout the course and should not proceed from one learning unit until training staff consider that they have met the required practical level of competency, with particular attention being paid to the ability to collect a blood sample, prepare a thick and thin blood film, and blood film staining with Giemsa.

Final Assessment

Each trainee should be assessed at the end of the course. This examination should focus on their ability to distinguish between negative and positive slides, identify the parasite species and grade or calculate parasite density in accordance with their skill at the time of the examination. This should be based on a minimum slide set, a model of which is given in Box 2.

Box 4 Minimum slide set for competency assessment of peripheral level microscopists

- 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;
- 1 mixed slide containing *P. falciparum* and either *P. vivax* or *P. ovale*.

6.2.4 Minimum grades of competency

Table 9 lists the minimum competency levels that should be achieved by microscopists working at the peripheral level.
Table 9. Minimum competency levels for peripheral level microscopists

<table>
<thead>
<tr>
<th>Competency</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity – parasite detection</td>
<td>90%</td>
</tr>
<tr>
<td>Specificity – species identification</td>
<td>80%</td>
</tr>
<tr>
<td>Accuracy of reporting <em>Plasmodium falciparum</em> when present</td>
<td>95%</td>
</tr>
<tr>
<td>Quantitation – accurately distinguishing <em>Plasmodium falciparum</em> at &lt;10/field and &gt;10/field</td>
<td>80%</td>
</tr>
</tbody>
</table>

### 6.3 Refresher training

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

- All persons performing malaria microscopy should have refresher training every two to three years;
- Refresher courses should be for a minimum of one week duration;
- Refresher courses should include more stringent training on species identification and, depending on the country, on quantitation; and
- The reassessment of a microscopists’ competency by examination every three to four years is considered extremely important.

### 6.4 Retraining

If a microscopists’ performance is considered to be poor based on slide validation and proven to be related to unsatisfactory competency, after validation of their work during consultative visits\(^\text{11}\), the following actions should be taken:

- additional consultation visits should be arranged for corrective training;
- the microscopist should be given 2–3 opportunities to improve;
- as appropriate, formal retraining should be provided (such as attending a further training course);
- the person’s eyesight should be checked if performance is poor.

If the person fails to improve after the above, then they should not be permitted to examine and report on malaria slides.

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\(^{11}\) All factors affecting performance need to have been considered (see Section 8 below for details),
The performance of malaria microscopy laboratories and their staff can be monitored by supervisory visits and by the external validation (cross-checking) of slides routinely taken for malaria diagnosis.
7.1 Planning the monitoring process

Both validation of slides and supervisory visits have distinct advantages and disadvantages, as well as varying resource requirements. It is unlikely that any country will be able to fully implement all of the methods without a step-wise approach that takes into consideration existing organizational structure, available resources, and staff proficiency at each of the collaborating laboratories.

An important step in any process to detect problems in performance is the application of appropriate strategies to correct them. Therefore, available resources to implement quality improvement are a critical consideration when designing a step-wise approach to QA. Resources will also be necessary for ongoing performance assessment to evaluate the success of problem-solving strategies.

The resource requirements for monitoring the performance of malaria microscopy laboratories and the essential steps in its implementation are summarized in Table 10 and Table 11.

**Table 10. Requirements for monitoring accuracy of laboratory/test centre results**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Personnel</th>
<th>Administrative</th>
<th>Technical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supervisory visits</td>
<td>Central level staff trained in all elements of QA for visits to intermediate laboratories.</td>
<td>Adequate funds to cover travel of staff from national or intermediate level to the peripheral level.</td>
<td>Checklist of activities to be carried out during supervisory visit.</td>
</tr>
<tr>
<td></td>
<td>Adequate numbers intermediate level staff trained in all elements of QA for visits to peripheral laboratories.</td>
<td></td>
<td>Standard monthly reporting form for use by peripheral laboratories.</td>
</tr>
<tr>
<td></td>
<td>Peripheral staff trained in principles and process of supervisory visits to smaller laboratories.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Validation of blood slides by cross-checking.</td>
<td>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.</td>
<td>Sufficient slide storage for the number of slides required for cross-checking.</td>
<td>Standard operating procedures for blind cross-checking of slides including instructions for slide sampling, standard reporting forms and details of the statistical basis of evaluations.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A reliable system for the dispatch of slides from the peripheral to the intermediate laboratory.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Effective communications system to deliver feedback of results from intermediate to peripheral laboratories.</td>
<td></td>
</tr>
</tbody>
</table>
### Table 11. Essential steps for planning for the monitoring process

<table>
<thead>
<tr>
<th>Step</th>
<th>Assess</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Carry out situation analysis</td>
<td>See Section 4.3, Table 3</td>
<td>See Section 4.3, Table 3</td>
</tr>
<tr>
<td>2. Plan specific steps for establishment or improvement of monitoring methods, including, as appropriate, a timetable for establishing minimal, intermediate and optimal level activities.</td>
<td>What are realistic short-term options for implementing or expanding the monitoring process? What methods fit best with available resources? Who are the most important partners to include in the implementation and improvement process? What is the priority for implementing each action? Consider the current level of performance, if known, as well as any monitoring activities in place. In the initial stages of establishing monitoring activities, very little will be known about performance. Establishing a comprehensive country-wide cross-checking programme may take several years. Implementation should be step-wise.</td>
<td></td>
</tr>
<tr>
<td>3. Define and obtain the necessary resources.</td>
<td>Are additional resources available? What are potential resources for obtaining additional staff, equipment and microscopes, and supplementary funds? What is the timetable for obtaining new resources? What data is required to support the need for additional resources? Planning should attempt to minimize the gap in time between available and required resources. Long term planning may be necessary to obtain adequate resources to implement fully the monitoring programme at the optimal level. It is strongly recommended that SMART indicators are developed in funding proposals (see Section 13).</td>
<td></td>
</tr>
<tr>
<td>4. Carry out pilot project and document results.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Evaluate and modify plan based on results of the pilot project.</td>
<td>Plan should be modified according to availability of resources. Particular attention should be given to feasibility of workload and to issues of validity of control measures.</td>
<td></td>
</tr>
<tr>
<td>6. Assess impact.</td>
<td>Has corrective action resulted in improved action? Improvement over time indicates that methods are feasible and effective.</td>
<td></td>
</tr>
<tr>
<td>7. Modify and expand plan as required.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
7.2 Supervisory visits (on-site evaluations)

Supervisory visits to peripheral diagnostic facilities are important to strengthen communication between the different levels of the QA programme, to identify the reasons for poor performance and to recommend the appropriate actions to correct these deficiencies.

However effective use of supervisory visits require:

- adequate human and financial resources;
- good interpersonal skills—there will be variable effectiveness according to the skills of the person conducting the consultation visit;
- adequate time for the visit (at least one day);
- a comprehensive plan to provide structure for comparing activities and performance problems from one visit to another, especially if they are performed by different supervisors;
- recording and reporting observations made by the supervisor; and
- effective follow-up and action to correct deficiencies.

7.2.1 The need

Supervisory visits are likely to be the most effective form of supervision of a programme, and can also provide an opportunity for correcting poor performance identified by cross-checking of slides in more developed programmes.

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

- personal problems such as family issues or sickness;
- poor motivation for a variety of reasons;
- poorly maintained microscope;
- poor quality or incorrectly stored reagents;
- stock-outs of reagents or other essential items;
- poorly prepared blood slides;
- poorly stained blood slides;
- poorly labelled blood slides;
- excessively high workload; and
- clerical errors.

Most of these deficiencies can be effectively identified by a supervisory visit.

It may not be possible to carry out supervision in all situations. For example, supervision may not be practical in some regions either due to the remoteness of the facilities to be visited or because of the unavailability of staff. Consideration must also be given to the fact that the authority of the supervisory staff may, in some circumstances, be adversely affected by a variety of factors, including gender, age and internal country tensions between different groups.
Chapter 7: Supervision of Malaria Laboratories

7.2.2 Frequency of visits

Supervisory visits should be carried out routinely and regularly, at a minimum of every six months and preferably with greater frequency.

During the initial establishment of the QA system, it may be necessary for more frequent visits. Special visits may also be necessary if problems are identified during the external cross-checking of blood slides submitted to the intermediate level. In those countries where health sector reform has been instituted, these visits should be integrated with the evaluation of general health services and laboratory quality assurance activities for other laboratory diagnosed diseases.

Sufficient time must be allotted for the visit to include observation of all aspects of the work associated with malaria microscopy, including the preparation, staining and reading of slides, the adequacy and safety of laboratories, the workload of laboratory staff and the adequacy of equipment and supplies.

The staff of the facility to be evaluated should be informed in advance of the supervisory visit. However, unannounced visits may take place may be conducted dependent on conventions in individual countries. Microscopists should have the opportunity to discuss cross-checking results and feedback from the supervisors. The report of the supervisory visit should be sent promptly to the microscopist (i.e. within 14 days).

7.2.3 Expected outcomes

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

- carry out validation of routinely taken slides by cross-checking;
- correct incorrect procedures on-site;
- relate the conditions of work to the performance of the staff that has been assessed by independent cross-checking of slides;
- assess the internal quality control procedures and the logistic procedures for maintaining equipment and supplies;
- ensure availability of SOPs, bench aids and other reference materials;
- discuss with microscopists and laboratory management problems encountered by the laboratory and make improvements “on-the-spot”;
- make decisions on training and retraining;
- build up communication with the staff in the routine laboratories; and
- ensure retraining if indicated by pervious performance or needs.

7.2.4 Check list for tasks to be carried out

Every QA programme will need to develop checklists to assist laboratory supervisors during the consultative visits and to allow for the collection and analysis of standard data

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12 This will be essential in the early stages of development of the QA programme when the capability of external QA by intermediate laboratories may not be possible due to inadequate resources.
for subsequent remedial action. Each country must also establish a standard definition of what is acceptable for each checklist item, based on the guidelines established by WHO and the resources available in the area. An important component of using any checklist is to provide sufficient training and standardization so that the checklists are used consistently. Programmes may need to revise the checklists in the light of problems that are frequently identified during such visits.

A comprehensive list of all operational elements to be observed will help to ensure consistency in laboratory evaluations and provide immediate feedback to the microscopists to facilitate rapid corrective action, as well as serve as documentation of the visit and record of current conditions and actions needed. An example of a reporting form for on-site evaluation is provided in Annex 4.

The form should be completed during the visit and discussed with the microscopists before the supervisor leaves the clinic. The results and need for corrective action or additional resources should be reported both to the NMCP and the head of the laboratory being visited.
Chapter 8

Cross-checking Malaria Slide Results

The basic principles of slide cross-checking (validation) are that:

- validators should be subject to a regular external competency assessment to maintain their skills and credibility;
- there should be a fixed number of slides checked;
- ideally, slides for cross-checking should not be selected by the same personnel who carry out routine malaria microscopy at the centre being evaluated (if this is not possible, a verifiable systematic method should be used to minimize bias); and
- cross-checking must be performed blindly—the person performing the cross-check must not know the results at the time of slide reading.
Cross-checking aims to detect major deficiencies in laboratory performance requiring urgent rectification. These may be due to poor competency, poor equipment, poor reagents, or poor infrastructure and work practices.

Cross-checking performance is essential in a malaria microscopy programme, although it should be seen as complementary to the process of competency assessment and retraining. Cross-checking at a validation centre may be replaced by regular supervisory visits that include cross-checking at the technician’s workplace. This is preferable but requires significant resources. Cross-checking must always be coupled with a system for assessment and correction of poor performance, including re-training and a method to address the other factors affecting performance (outlined in Chapter 7 Section 7.2.1).

Traditional approaches to cross-checking have tended to require high personnel resources due to the large number of blood films requiring validation. This chapter provides a recommended method for a sustainable cross-checking programme that provides a high probability of detecting major deficiencies in performance.

### 8.1 Requirements for cross-checking

Cross-checking of routine blood slides by a validation centre can be highly demanding on human and financial resources and requires that:

- both microscopists and their supervisors are adequately trained in the principles and operation of the cross-checking system;
- an efficient logistics system network is established to ensure the selection of slides at the periphery and their transport to the supervisory level for cross-checking or storage if cross-checking will be done by the visiting supervisor;
- both microscopists and their supervisors are motivated and well-organized in the operations of the system;
- there is adequate budgeting and availability of funds to implement the system;
- microscopists send the slides to the supervisory laboratory at the designated times and understand the reasons for sending them;
- there is good communication between the supervisor and the technicians;
- there is prompt feedback of results by the supervisor so that action can be taken to correct errors as late feedback loses impact and is discouraging for the technicians;
- the number of blood films cross-checked must be large enough to provide a fair assessment of a technician’s performance, but small enough to be sustainable, not imposing too heavy a burden on the programme.

As validators at mid-level and reference laboratories have other duties, it is likely that they will only have four or five days per month devoted to cross-checking. Because the QC slides will be either negative or with low parasite densities (see Section 8.3.6) they will require at a minimum of 6 minutes to cross-check and therefore blood film analysis can only be carried out on at most 50 slides per day. A single validator is therefore limited to cross-checking a maximum of 20 to 25 lower-level readers.
Chapter 8: Cross-checking Malaria Slide Results

8.2 Principles of cross-checking and classification of errors

External quality assessment by cross-checking relies on a blinded re-examination of the selected sample of slides by staff from a higher-level laboratory. The validator performing the re-examination must have a high level of skill in malaria microscopy, have a thorough understanding of the sources of errors, and be trained in compiling the summary report that will eventually be returned to the peripheral laboratory (and the NMCP). It is essential that the rechecking is performed by an accredited malaria microscopist of proven competency. 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. This information may be very useful to supervisors responsible for providing feedback to the peripheral technicians, assessing possible reasons for high false-positive or false negative results, and implementing plans for retraining and corrective action.

Ideally microscopists should be validated individually but in laboratories with a large number of microscopists, this may be impractical on a regular basis. In such cases the laboratory as a whole may be validated with individual microscopists validated internally by the head of the laboratory as required. The organization of the validation process is given in Figure 3.

Figure 3. Organization of the validation process
Cross-checking must be blinded to ensure objectivity. The validator rechecking the slide must not know the initial result. Once a slide set is read, and discrepant results are identified (differences between the clinic microscopist and the validators), the validator should re-check the discrepant slides with a further un-blinded reading to confirm that they did not make an error, before reporting the result as discrepant.

Whenever there is a discrepancy between the reported result and that found in the rechecking process, the peripheral laboratory must be informed as soon as possible. The controlling laboratory should give feedback where appropriate that includes likely explanations for the discrepancy as well as suggestions for corrective actions. Results should be recorded in a database and this must be available to the supervisor before the next supervisory visits, during which discrepant results and the likely reasons for them must be discussed. Where constructive feedback and supervision are an integral part of the cross-checking process, considerable improvement in laboratory performance can be seen (Figure 4). Common causes for errors detected by slide rechecking are shown in Table 12.

Figure 4. Improvement in cross-checked laboratory performance in a cohort of laboratories in resource-limited settings, 2005–2007

Source: MSF Holland
### Table 12. Common causes of errors in blinded slide rechecking

<table>
<thead>
<tr>
<th>Possible causes</th>
<th>Notes</th>
<th>Suggested actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low parasite density</td>
<td>There is a 34.8% probability of a false negative result at a parasite density of an average of one parasite per 100 fields. Since the validator should perform a second examination if there is a disagreement with the initial reported result, this will mean that the validator will read at least 200 fields. Even at 200 fields there is a 10.7% chance that 1 parasite/100 fields may not be detected. Because the QC Protocol specifically selects slides with a low parasite density for crosschecking, this problem may occur from time to time.</td>
<td>Nil</td>
</tr>
<tr>
<td>Stain faded since original examination</td>
<td>Can be minimized by (1) laboratories using high quality stains and (2) ensuring crosschecking is performed with minimum delay.</td>
<td>Re-staining ‘false negatives’. The validator should assess the staining quality of other components (such as platelets) to assess staining quality. However re-staining is usually only appropriate if stain fading is likely to have occurred.</td>
</tr>
<tr>
<td>Too high a QC workload for the validator</td>
<td>One of the principal reasons for the small sample size of the QC Protocol is to manage the workload of reference laboratories and other validators.</td>
<td>Reduce the workload of the validator.</td>
</tr>
<tr>
<td>Low skill level by the validator</td>
<td>The QC Protocol is based on the premise that the crosschecking is performed at a high standard.</td>
<td>A major problem – consider changing the validator.</td>
</tr>
<tr>
<td>Pressure on laboratory staff by clinical staff to find malaria parasites when there is a clinical suspicion of malaria.</td>
<td>Some clinical staff can be critical of laboratories (and assume poor quality slide examination) that report negative findings in patients with symptoms consistent with malaria. Since clinical officers have a higher status than laboratory staff, this pressure can potentially be very strong.</td>
<td>The clinical staff should be fully aware of the laboratory QC results – if the QC results are good then the clinical staff should respect the results of the laboratory. Because low parasite densities can be difficult to detect on occasions it is correct for a clinician to request a re-examination in some cases. However the results of a laboratory with good QC results should be respected.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Possible causes</th>
<th>Notes</th>
<th>Suggested actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory staff choosing to report negative slides as ‘weakly positive’ because they believe this is ‘safer’.</td>
<td>A major problem that can be caused by either (1) lack of skill or confidence or (2) pressure from clinicians.</td>
<td>Retraining to increase the skill and confidence of the laboratory staff. Assessment of the programme to identify any external causes of the problem (such as pressure from clinicians to report positive findings).</td>
</tr>
<tr>
<td>Artifacts such as stain deposit, non-filtered water, incorrectly interpreted as malaria parasites.</td>
<td>Common causes – staining with dilute Giemsa stain &gt;15 minutes after preparation; using tap water to prepare buffer; using poorly cleaned slides; fungus contaminated slides.</td>
<td>Prepare diluted Giemsa stain immediately before use. Always use filtered or bottled water for preparation of buffer. Use only new slides or slides that have been fully cleaned. Never use slides that have become contaminated by fungus. Retraining of staff in good laboratory technique and recognition of artifacts.</td>
</tr>
<tr>
<td>Howell-Jolly bodies and platelets misidentified as malaria parasites</td>
<td>Caused by poor reading skill. Platelets are less of a problem as laboratory staff are familiar with their morphology. Laboratory staff can be less familiar with Heinz bodies</td>
<td>Retraining</td>
</tr>
<tr>
<td>Poor skill levels by laboratory staff.</td>
<td>Includes all the above</td>
<td>Retraining</td>
</tr>
<tr>
<td>Clerical error</td>
<td>Can occur at any time. The Clerical Error Index provides an 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.</td>
<td>Dependent upon the Clerical Error Index. If the Clerical Error Index is unsatisfactory then the laboratory clerical systems need to be reviewed and improved. If the Clerical Error Index is satisfactory then no action is indicated.</td>
</tr>
</tbody>
</table>
8.3 Protocol for slide cross-checking

The QC protocol is based on the following:

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

See Annex 5 for details on the principles on which the protocol is based, its advantages and its limitations.
8.3.1 Overall objectives

The overall objectives of this protocol are to:

- provide a standardized methodology for malaria microscopy that is simple to use, easy to understand and uses a minimum QC sample size;
- allow the development of realistic minimum standards based on actual performance data;
- enable benchmarking; and
- promote a high standard of laboratory testing.

8.3.2 Scope

This protocol is applicable for laboratories and test centres performing routine diagnostic malaria microscopy.

The small sample size is not applicable for the quality control of blood slides taken for research purposes such as clinical trials of new drugs and potential vaccines and monitoring antimalarial drug resistance.

8.3.3 Slide storage

All routine slides examined and reported by the laboratory must be stored in secure slide boxes protected from excessive heat and/or humidity until the QC slides have been selected.

Slides must be stored systematically so there is a direct link between the results in the laboratory register and the slide location\textsuperscript{14}, such as divided into positive and negative slides or sequentially, according to the date on which they were processed.

Routine slides must not be discarded until the QC slides have been selected.

8.3.4 Sample selection from the laboratory register

Quality Control is critically dependent on the correct selection of the QC sample and therefore the sample selection protocols detailed for each test must be strictly followed.

The three critical requirements are the method of selection (random or systematic without 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 the slide storage boxes.

\textsuperscript{14} Slides however can be stored in the most convenient way for the individual laboratory – either divided into positive/negative or all slides sequentially.
Chapter 8: Cross-checking Malaria Slide Results

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

**8.3.5 Sample size**

The required minimum QC sample size is 10 slides.

**8.3.6 Recommended sample selection criteria**

It is the responsibility of the laboratory supervisor to randomly select for QC the minimum 10 slides each month (5 reported as low-density, 5 reported as negative) using a random numbering system.\(^\text{15}\) It is important that QC slides must be selected randomly from routine tests performed during the calendar month or a shorter period (see below). Therefore, routine slides must not be discarded until the QC slides/samples have been selected.

Five weak positive slides with parasitaemias of 20–200 trophozoites/µL and plus 5 negative slides should be selected. Slides with parasite densities greater than 200 trophozoites/µL should not be selected.

This protocol preferentially cross-checks weak positive thick blood films to maximize the statistical power of a small sample size. Cross-checking strongly positive blood films provides minimal information because:

- even poorly skilled microscopists can generally detect strongly positive slides with relative ease, therefore these slides have low analytical value in 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).

Further strongly positive blood films often represent a significant majority of the positive slides examined by laboratories. A random selection of all positive slides will therefore include insufficient weak positive slides to provide a meaningful measure of performance. (For example, if 60% of blood films in a programme are strongly positive, then a random selection of 5 positive slides is likely to contain 2 or less weakly positive slides. This is too few to be informative).

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, and all negative slides.

In laboratories with a high workload, where a minimum of least four to five weak positive slides will be available during each week, weekly QC sampling may be carried out according to the following strategy:

\(^\text{15}\) If it is not possible to generate a random number system, selection should be based on random or systematic sampling independent of the microscopist(s) being checked.
- Week 1 – randomly select 2 weak positives and 1 negative slide
- Week 2 – randomly select 1 weak positive and 2 negative slides
- Week 3 – randomly select 2 weak positives and 1 negative slide
- Week 4 – randomly select 1 weak positive and 2 negative slides.

Depending on the availability of transport, slides may be collected weekly, grouped and dispatched monthly to the validators.

### 8.3.7 Cross-checking

All 10 slides should be cross-checked for the presence and absence of parasite stages and for the accuracy of the differentiation of *P. falciparum* and non-*P. falciparum* parasites.

The use of five low positive slides per month (i.e. 20 slides in a 4 month cohort) limits statistical validity for species identification. However, it is much better to thoroughly cross-check 10 slides than to inadequately cross-check a larger number. However, laboratories are encouraged to perform more QC than the minimum requirement provided that there is sufficient capacity for all QC slides to be accurately cross-checked.

(i) **Time frame**

- Cross-checking should be performed as soon as possible after the end of each month and the results reported optimally within 2 weeks.

When, for practical reasons, it is not possible to meet these time limits, a longer time period may be acceptable. However, this must be for reasons of necessity and not convenience and **must** be approved by the quality control supervisor. An important principle of the QC Protocol is that QC results are an integral part of laboratory management and need to be available and analysed as soon as possible.

(ii) **Selection of the cross-checker (validator)**

Quality control is critically dependent on the accurate cross-checking of the QC slides.

The validator/cross-checker should be selected from the following personnel (listed in order of preference):

- a suitably trained member of the National Reference Laboratory;
- a similarly skilled member from the provincial/regional laboratory; or
- the laboratory supervisor or an experienced laboratory staff member appointed by the laboratory supervisor (internal cross-checking).

All should have had their competency assessed, and achieved the required standard, within the previous 3 years.\(^{16}\)

\(^{16}\) This method should only be used as a second preference when slides cannot be referred to a reference laboratory.
The validators must in turn be enrolled in an EQA programme, with some form of internal or external cross-checking.

It is important that, when QC cross-checking is performed internally, the identification details on the slides must be concealed and all slides identified only by an internal QC number. The person cross-checking the slides must not know the identity of the slides. The person selecting the QC slides should not be the same person who will do the cross-checking of the slides.

(iii) Accuracy of cross-checking

Slides must be cross-checked with considerable care. It is to be expected that the level of accuracy of cross-checking will be higher than for routine slide reading and therefore cross-checking may on occasions detect weak positives undetected in routine work. This should not be considered a criticism of the person who performed the routine examination, unless that person repeatedly reports false negative results. A reduced sensitivity in routine examination is more frequently caused by variables such as high workload and poor equipment, not a lack of skill by the reader. The intention is for the cross-checking to approach a gold standard level as closely as possible.

8.3.8 Recording of results

All results should be recorded in a 2x2 table as follows:

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

<table>
<thead>
<tr>
<th>Routine lab. Result</th>
<th>Validator – cross check</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>B</td>
</tr>
<tr>
<td>Negative</td>
<td>A</td>
<td>C</td>
</tr>
</tbody>
</table>

Where:

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

\[
\text{% Agreement} = \frac{(A + D) \times 100}{A + B + C + D}
\]
(ii) QC monitoring based on the monitoring the accuracy of the differentiation of *P. falciparum* and non-*P. falciparum*:

<table>
<thead>
<tr>
<th>Validator – cross check</th>
<th>Laboratory</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. falciparum</em> present</td>
<td><em>P. falciparum</em> NOT present</td>
<td></td>
</tr>
<tr>
<td><em>P. falciparum</em> present</td>
<td>A</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td><em>P. falciparum</em> NOT present</td>
<td>C</td>
<td>D</td>
<td></td>
</tr>
</tbody>
</table>

Where:

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

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

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

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

\[
\% \text{ Agreement} = \frac{(A + D) \times 100}{A+B+C+D}
\]

### 8.3.9 Statistical analysis

QC results should be analysed using monthly analysis and progressive four month cohort analysis.

The analysis and reporting of the results of cross-checked slides should be in a standardized format to avoid misunderstanding between validators and those whose performance is being checked.

To be meaningful—and since they may have considerable influence on staff morale and even on employment—results must be robust so that chance plays only a small part. Therefore, the threshold rate for incorrect results that should trigger corrective action must be sufficiently different from that expected of a reasonable microscopist or laboratory. This threshold requires benchmarking—that is, determining what constitutes a reasonably achievable standard by comparing many results. The poor performing laboratories can then be identified and remedial help provided for them (by addressing competency, deficits in materials and/or workplace conditions). The system described above provides a representative sample for performing a slide validation scheme. Experience with this method has achieved a mean performance of 97.4% of slides interpreted correctly.

(i) Monthly analysis of QC results

Individual monthly results should be evaluated for any major errors. This is important as it allows for rapid feedback of the development of any significant problems.
However, because of the small sample size, the result may not necessarily reflect the true overall performance of the laboratory for at least the following reasons:

a. There may have been an unusual event during a particular month such as an exceptionally high workload, a reagent problem or a new staff member. These situations must be reported centrally.
b. Because errors are not necessarily evenly distributed and there may have been more errors than normal during a single month this may be balanced by a lower than normal error rate in another month.
c. The limitation of a sample size of 10 is that single errors significantly affect the calculated Percentage Agreement. Hence a single error in a cohort of 10 QC samples will reduce the Percentage Agreement to 90%.

The interpretation of individual monthly results needs to take into account the previous performance of a laboratory or test centre. As a guideline:

**When the previous QC results have been good to satisfactory**
- 2 errors out of 10 results is an alert
- ≥3 errors out of 10 results require immediate investigation

**When the previous QC results have been poor**
- A result that is better than the 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.

(ii) Progressive four month analysis

Progressive cohort analysis calculates the % agreement, % false-positives and % false negatives on the last 4 months of QC results. That is to say:

After 4 months — use data from months 1,2,3,4
After 5 months — use data from months 2,3,4,5
After 6 months — use data from months 3,4,5,6

**Rationale**

The disadvantages of individual month analysis can be addressed by analysing QC data over longer periods of time, such as in 3, 4, 6, 12 month cohorts. For example while a single error will reduce an individual monthly result to 90% agreement, in contrast a single error will only reduce a 4-month cohort result to 97.5%. This allows more accurate assessment of the actual laboratory performance

However there needs to be a balance between the greater accuracy of analysing a larger sample – for example analysis of six months data will be more accurate than three months of data and the greater efficiency of a smaller sample to detect recent changes in laboratory performance.17

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17 There is limited value in determining the performance of a test centre in the distant past. Circumstances may have changed significantly since that time.
Calculation of 4 month indices

4 month % Agreement = \[ \frac{\% \text{ Agreement of months 1+2+3+4}}{4} \]
4 month % False-positives = \[ \frac{\% \text{ False-positives of months 1+2+3+4}}{4} \]
4 month % False Negatives = \[ \frac{\% \text{ False negatives of months 1+2+3+4}}{4} \]

Interpretation

a. **Benchmark analysis** – It is recommended that QC benchmark standards are set annually and take into account both the actual performance of field testing as determined by the QC Protocol, and the overall goals for the accuracy of testing required to support effective clinical diagnosis.

b. **Quartile analysis** - to enable individual test centres to assess their performance relative to the performance of other test centres, QC data is centrally analysed 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 the 75% of test centres
4th Quartile - the highest result achieved by any one or more test centre

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

**Example:**

Based on a laboratory performing QC on 10 samples/month

<table>
<thead>
<tr>
<th>Month</th>
<th># errors</th>
<th>Monthly % agreement</th>
<th>Progressive % agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>0</td>
<td>100%</td>
<td>Too few samples</td>
</tr>
<tr>
<td>Feb</td>
<td>1</td>
<td>90%</td>
<td>Too few samples</td>
</tr>
<tr>
<td>Mar</td>
<td>0</td>
<td>100%</td>
<td>Too few samples</td>
</tr>
<tr>
<td>April</td>
<td>1</td>
<td>90%</td>
<td>95%</td>
</tr>
<tr>
<td>May</td>
<td>3</td>
<td>70%</td>
<td>85.5%</td>
</tr>
<tr>
<td>Jun</td>
<td>0</td>
<td>100%</td>
<td>90%</td>
</tr>
<tr>
<td>July</td>
<td>1</td>
<td>90%</td>
<td>87.5%</td>
</tr>
<tr>
<td>Aug</td>
<td>0</td>
<td>100%</td>
<td>90%</td>
</tr>
<tr>
<td>Sept</td>
<td>1</td>
<td>90%</td>
<td>95%</td>
</tr>
<tr>
<td>Oct</td>
<td>0</td>
<td>100%</td>
<td>95%</td>
</tr>
<tr>
<td>Nov</td>
<td>1</td>
<td>90%</td>
<td>95%</td>
</tr>
<tr>
<td>Dec</td>
<td>0</td>
<td>100%</td>
<td>95%</td>
</tr>
</tbody>
</table>
Chapter 8: Cross-checking Malaria Slide Results

This demonstrates that 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 a progressive or a fixed-period cohort analysis is used. Using the above data, the same distortion occurs when the data is analysed in three fixed periods of time, for example:

Jan. – April insufficient data
May – Aug. 90%
Sept. – Dec. 95%

This distortion can also apply in the opposite direction, when an atypically good performance in one month can overestimate the actual laboratory performance for several months following the event.

This effect must be taken into account when analysing QC results.

(iii) Calculation of the true false-positive rate

The calculation of the true false-positive rate is based on the assumption that strong positive slides have a very low probability of being false-positives. If a blood film is reported by a laboratory as being strongly positive and found to be negative on cross-checking, then this most probably represents a clerical error rather than a technical reading error.

To calculate the true false-positive rate individual laboratories and test centres need to record the proportion of strong and weak positives reported in the analysis period.

The true false-positive rate =

\[ \frac{\text{QC false-positive} \times \text{total number of weak positive blood films}}{\text{Total number of positive blood films}} \]

Example:

Over a 4-month period a laboratory reports 500 positive blood films, including 450 strong positive and 50 weak positive results. During this same period 20 weak positive thick films are randomly selected for cross-checking (5 weak positives each month) and 2 of these blood films are found to be negative.

- QC false-positive rate = 2/20 = 10%
  - The total number of weak positives in this period = 50.
- Therefore, 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 alternatively clerical errors)
- Total positive slides in this period = 500.
- Therefore, the calculated true false-positivity rate = 5/500 = 1%
(v) **Benchmarking**

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

- Central reporting and analysis of results
- Feedback of comparative data to the participating laboratories and test centres.

**8.3.10 Reporting results**

Monthly QC results should be must be reported to the QC supervisor optimally within 2 weeks from the end of the calendar month in which the routine testing was performed. Results should be reported using a standardized quality control reporting form.

Model forms for reporting the results of cross-checking of slides by the external validator are given in Annex 6 and Annex 7.

**8.4 Action to be taken in the case of discordant results**

When cross-checking is performed by a reference laboratory or by a laboratory supervisor with a higher level of skill than the routine laboratory staff, the cross-check result should generally be considered to be the correct result. However, if the laboratory staff who performed the initial testing believes that the cross-check result is incorrect, then they should be given the opportunity to re-examine the slide or sample. Microscopy slides sent to a reference laboratory for cross-checking should, if practical, be returned to the routine laboratory after examination.

When cross-checking is performed by persons with a similar level of skill to the routine staff who performed the initial testing, then any discrepancies should be reviewed by the original laboratory.

When a discrepant result is subsequently reviewed by the laboratory that performed the initial reading the following actions should be taken.

- If the laboratory that performed the initial reading agrees with the cross-check result (that is to say the laboratory agrees that there was an error in the original reading), then 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 cross-check result, then the slide/sample should either be re-examined by the cross-checker or referred to a third reader for resolution. If the cross-check result is found to be in error then the original result is recorded as a correct result in the QC analysis.
Chapter 9

Reference Slide Sets For Training (Setting up a national slide bank)

The provision and maintenance of a set of high quality and well characterized malaria reference slides is an essential part of all national QA programmes to support of the training of microscopists and accreditation of their expertise.
9.1 The need

The need to improve the competency and performance of malaria microscopists has increased the demand to produce well characterized and high quality national reference slide sets for the continuous training and assessment of basic malaria as well as expert microscopists, who will become the managers and supervisors of the national QA programme.

Normally, a national programme should develop its own slide bank to support its QA programme. Regional slide banks may be set up by multi-country health organizations, research institutions or similar bodies. They should supply well-characterized malaria blood films to support national-level retraining and assessment programmes, such as the programme outlined in Chapter 6.

9.2 The constitution of a national slide bank

National slide banks should contain, as a minimum, slides of all the malaria species currently found in the country, as well as blood slides that have been confirmed as malaria-negative. When an effective system is in operation and dependant on available financial and human resources, consideration should also be given to the inclusion of slides of:

- Local zoonotic species that may have affected individuals or communities such as *P. knowlesi* in parts of Southeast Asia; and
- Other blood parasites common to the country, e.g. microfilaria, trypanosomes, *Borrelia* species.

The number of slides of each category should be based on the relative parasite prevalence encountered by the national programme. The size of the bank must be determined by a needs assessment, taking into consideration:

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

A policy on access to the bank will need to be developed to achieve this.

The species diagnosis of all slides should be expertly determined and confirmed in a reputable laboratory, wherever possible, by polymerase chain reactions (PCR) techniques.

9.3 The aims

The aims of the national slide bank are to provide:

- Sets of “known, replicate” slides for QA training in malaria microscopy and quality assurance (QA);
Chapter 9: Reference Slide Sets for Training

- A permanent reference collection of the malaria species present in the country; and
- Sometimes, sets of reference slides on request from outside the country.

However, the feasibility of achieving all these objectives and goals 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 these objectives and how they will be met.

9.4 Plan of action

Past experience shows that the production of slide banks is not sustainable without an effective plan of action, adequate funding, and the commitment of trained laboratory and medical staff. It also requires ethical clearance for the collection of the samples, high-quality supplies and reagents, careful slide preparation, excellent logistic and laboratory support and an efficient archiving system that allows the practical retrieval of data and slides for their dispatch and replenishment.

Careful planning of slide bank activities is, therefore, critical to success.

The National Reference Laboratory (NRL) should be responsible for the establishment and maintenance of the national slide bank and the development of the plan of action for its production and maintenance.

The following are the essential steps of such a plan of action:

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

The specific objectives of the slide bank, its content, production and use should be met should be determined by NRL staff in consultation with all colleagues responsible for training and supervision within the QA programme and the NMCP.

The essential steps in establishing a national slide bank are given in Figure 5 and the detailed standard operating procedures in Annex 8.
Figure 5: Flow chart of essential steps for establishing a national slide bank

- Justify need
- Develop plan of action, and policy on access
- Identify resources
- Develop database
- Finalize SOPs
- Prepare materials and equipment
- Collect specimens, make and stain slides
- Confirm quality of slides and store
- Identify targets: Number of slides, Parasite species, Parasite densities
- Decide selection and collection methods
- Estimate costs
- Develop job descriptions
- Contact collaborating centres and collection sites, and develop agreements
- Obtain ethics approval
9.5 Costing

The establishment and operation of a slide bank facility requires adequate and sustainable funding but, if planned and implemented carefully, the funds required can be modest. 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 acceptable levels.

The largest financial outlay will probably be for:

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

The essential equipment and supplies should include the following:

- 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 frosted end
- Cover-slips for mounting blood films, various sizes
- Detergent
- Micropipettes
- Drying cloths for slide drying
- Disposable syringes (5 ml. capacity) and needles
- EDTA treated collecting tubes (5 ml. capacity)
- Latex protective gloves (unpowdered)
- Stain and reagents such as buffer salts (or tablets)
- Desiccators and active (indicator coloured) silica gel
- Record forms (coded), registers
- Objective marker, diamond-tipped (optional)
- A digital camera and attachment, for the microscope (optional)

9.6 Selection of staff

The responsibility for the organization, planning and implementation of slide bank activities should rest with at least one senior laboratory technologist (the title may differ country to country) ideally working within the NRL. This technologist should be highly skilled in all the activities of malaria parasite diagnostics and with proven abilities to organize and oversee a small team of personnel who have been trained in slide bank operations.

The senior laboratory technologist will need to be assisted by at least two junior staff each working full time for the period of establishing the bank. Additional staff that may be required during field or collecting activities but these can be seconded, and participate as needed, following previous agreement between units or departments.
It may be necessary to modify the existing job description of some or all of the above staff and, if necessary, identify the additional training needs that will be required before implementation of slide bank activities. Both the change in job description and the additional training may be minimal but it is essential to carry out both so that all staff, who are involved, are fully aware of their additional responsibilities and able to perform them effectively. Dedicated staff with specific training are essential.

9.7 Methods for slide collection

Slides can be collected by hospital or health centre-based activities or by selective field surveys. Staff should be aware that each method has specific advantages and disadvantages. Careful considerations of the “pros and cons” of each approach should be made but the final decision about which is the most appropriate can only be based on local knowledge.

9.7.1 Hospital or health centre

This system relies on donor selection from referred patients or patients reporting to a health facility’s outpatient department.

Activities can be organized in the following ways:

(a) **Resident staff select the donor during the daily routine and inform the slide bank staff who travel to the hospital and take and process the blood sample.** Providing the collection centres are easily reached, this system works extremely well.

(b) **Resident staff cooperate during routine activities to select a suitable donor, collect the specimen and forward it rapidly to the slide bank centre for further processing.** The timely dispatch of blood specimens and receipt by the slide bank staff is critical as EDTA treated blood has about one hour at room temperature before parasite morphology starts diverging from the norm. If these requirements can be assured, this can be a successful approach.

(c) **Slide bank staff, temporarily based at the hospital, actively select suitable donors and carry out the whole production process.** This is an efficient approach but requires a steady supply of donors to be cost-effective. Space restrictions in smaller health centres can be a challenge 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 screen patients and any suitable cases referred to the team for blood collection before treatment is given.

9.7.2 Selective surveys

National malaria control programme surveys, or combined health activities in small communities, offer great advantages for collecting slide bank material. The chances of finding donors increases and can be highly cost-effective. Previous experience is essential.
to select the best time and the most potentially productive areas. Communities with high malaria prevalence are often far from available 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.

Establishing an operations centre allows a wider area and population to be covered so working and living facilities must be considered. Such regular activities provide materials sufficient for the routine needs of the slide bank and makes replenishment and updating fairly easy.

9.8 Selection of donors

Selection of donors is the most important activity in establishing a slide bank. It should be based on a set of criteria developed by the NMCP and in accordance with the highest ethical standards. The likely pathology to be encountered by the technicians in the country must be clearly understood before selection can start.

It is important to confirm the criteria governing donor selection before implementation including the following:

- Sampling method for the community and case selection criteria
- Minimum age for participating donors
- Parasite stages and species required
- Inclusion of parasite species as single or mixed infections
- Range of parasite densities
- Inclusion of unusual parasite forms and other blood parasites

Specific exclusion criteria should include:

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

9.9 Ethical considerations

Collections for blood banks should be based on certain basic ethical principles:

- All blood collections should be approved by an Institutional Review Board (IRB) designated to oversee research and specimen collections involving human subjects.

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18 Species, and parasite densities, are usually seasonally dependent. In hyper or holo-endemic areas, cases are available all year but densities increase in the high transmission season. The incidence of all species increases at this time, including *P. malariae* (and *P. ovale* when present). Therefore, a careful selection of the collection period ensures the greatest opportunities for detecting the required materials.

19 In malaria endemic countries, it may be necessary to ensure parasite negativity by the use of PCR or by sourcing specimens from non-endemic countries.
Donors should not be paid for providing medical specimens.

Informed consent must be obtained after the donor is made aware of 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 deal with malaria and other diseases likely to be encountered.

Each potential donor or their carer should be informed of the objectives of the programme, and the reasons and procedures for taking the blood sample. They or their carers or responsible person must sign a consent form confirming that they understand this request and agree to collaborate with the programme (a model Consent Form and Information Sheet are in Annex 9).

The risks and discomfort of blood donation are minimal. Donors may suffer pain at the phlebotomy site that may persist for a few days after blood collection. Potential complications of venepuncture include fainting, bleeding, bruising, haematoma formation or, very rarely, local infection. All precautions to minimize the risk of infection should be applied. The risk of 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 accessible only to relevant health project staff. All information accompanying banked slides should be completely de-linked from data identifying the individual by the use of a standardized 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.

9.10 Outline of slide collection and preparation

Slide preparation

Detailed SOPs for producing large numbers of uniform, high quality blood films are provided in Annex 8 of this manual. It is recommended that they be followed closely, to ensure that batches of microscopy slides produced at different times and at different sites are interchangeable. It is important to ensure that trainees or candidates under assessment are unable to determine the site of time of collection.

As stated previously, detailed planning is essential. The equipment lists provided must be adjusted for local requirements, and it must be ensured that all required equipment is available prior to commencing collections. The less delay in producing films after venepuncture, the higher the quality of the films is expected to be. It is vital to have a clear routine prepared from time of venepuncture to final labelling that will prevent any chance of
samples or films from different donors becoming confused. Batches should be maintained separately at all times.

Slide preparation should be run like a production line, with each technician involved in having a clear set of allocated tasks. Blood from a single donor should be processed at any one time (during film preparation and later staining), to prevent cross-contamination or mistakes in labelling.

Multiple templates for thick films should be made (Annex 11), and cleaned slides laid out on a workbench in rows to allow rapid, accurate pipetting. Thick films should be spread rapidly and carefully on the templates, ensuring consistency in thickness across the film.

Once stained and dry (Annex 8), a selection of slides must be labelled and put aside for validation. The remainder of the slides from each donor should be placed in sealed slide boxes clearly labelled with the case ID for labelling once validation is complete, or labelled on site using an appropriate ID. A bar code labeller should be considered to allow future readers to be blinded to the details of the case (below).

**Dilution to selected parasite densities**

Where certain parasite densities are not available in the community being sampled, it is possible to produce films of selected parasite density by diluting with parasite-negative donor blood. However, it is preferable to avoid this if possible, as complications such as clumping of cells may occur. A protocol for dilution blood can be adapted from the dilution procedure in SOP 3.2 of the WHO Methods manual for Laboratory Quality Control Testing of Malaria Rapid Diagnostic Tests\(^\text{20}\).

**Labelling**

Slides should be labelled on the bevelled area clear of the blood film and cover-slip. For clarity, printed labels should be used, using high-quality labelling paper that will adhere after years of use. A bar code label printer (and bar code reader) should be considered, to allow blinding of slides from future readers who are undergoing competency assessment. Removal and return of slides to the bank storage may then be recorded in the database using the bar code reader, making future operation of the bank and tracking of slides easier.

**9.11 Data management and entry of data**

Data management should be based on the use of standardized reporting forms for which a model is given in Annex 10. Names and other unique information on donors should not be kept in the permanent records. However, a temporary log relating the participants’ numbers to their name is useful during the period that the samples are collected, but this should be destroyed at the end of each day. Essential information relevant to the slide bank activities is given below.

---

Demographic data

- Subject number
- Age
- Gender
- locality
- History of malaria treatment
- History of travel - optional

Malaria microscopy results

- Positive or negative film
- Species present, i.e. *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax*, *P. knowlesi*
- Presence of gametocytes
- Single or mixed infections
- Parasite counts against 500 WBCs, parasitized RBCs per 5000 RBCs
- Parasite density per microlitre (determined from true WCC)
- Concordance/discordance of validation results - optional

Electronic data entry

A computer data base should be developed for the study and based on the following principles:

- Only authorized users should have access to the database using a user name and password;
- Entries should be done from the original reporting forms directly into the database;
- Double data entry and cross-validation, with discrepant results checked against original data.

Records should be backed up regularly.

9.12 Slide bank storage and maintenance

Slide set operation and maintenance is reasonably straight forward requiring little more than modest laboratory space with storage for the slide sets.

It must be clearly understood that slide bank activities provide a service for the different activities of the NMCP and has materials available on demand for training and EQA activities. Sufficient stocks of slides should be maintained to meet the annual estimated requirements (based on a yearly needs assessment) so that short falls of stock and delays in dispatch do not occur. This requires an effective archiving and reporting system which tracks despatched slides, current levels of stocks and monitors their shelf life over time.
Chapter 10
Self Monitoring of Laboratory Procedures (Internal Quality Assurance)

Internal quality assurance is a process of effective and systematic internal monitoring of work carried out in a laboratory.

Internal quality control is very important and should be carried out by laboratory staff to check their own performance and to ensure reproducibility and sensitivity of laboratory diagnoses. Its organization is the responsibility of the laboratory head, but all personnel must be involved. A technician working in isolation should also conduct internal quality control, but the number of checks that can be done are more limited. However, those that are feasible should be carried out routinely.

The following basic technical aspects are required to be regularly monitored:

- use of equipment, especially the microscope and its present condition;
- quality of reagents and stains;
- performance of laboratory staff in using and following accurately standard operating procedures; and
- detection and recognition of parasites.

The following routine is recommended:

(i) Each Day. Check the quality of the stain by staining one thin and one thick blood smear and assessing the quality of red cell staining to control the buffer quality, staining of white blood cells for nuclear staining and staining of granules, and staining if parasite chromatin and inclusions if available.

(ii) Each week. Joint review by all staff of difficult slides encountered during the week; rechecking of a number of slides from each technician by the head of laboratory or by cross-checking between staff.

Weak positive and negative slides should be selected for submission to external review by the reference centre at appropriate intervals, as described in Chapter 8.

Cross-checking within the laboratory should be organized in a non-intimidating manner, consisting of ad hoc checking of unusual or uncertain slides, and a structured blinded cross-checking followed by discussion between the validator and the technician being validated. In most laboratories, both senior and junior microscopists should be involved in this process as a team.
Additional operational issues that affect the quality and impact of malaria microscopy at the periphery need to be addressed. These include:

- timely reporting of results to medical staff;
- demand for microscopical diagnosis laboratory;
- coordination between medical and laboratory staff;
- timely reporting of results to reference laboratory;
- timely submission of slides for cross-checking by reference laboratory (if included in the national programme); and
- adequate maintenance of supplies and equipment.

Standard operating procedures for all laboratory tasks, including those for internal quality control, need to be developed and be readily available in the laboratory. Strict control of these techniques and the equipment, as well as training of staff in their use, should be assured, and regularly reviewed (e.g. 3 monthly) in a formal, structured way (Annex 12). The internal quality control procedures need to be regularly checked during visits by technical staff from supervisory laboratories. Trouble-shooting guides for equipment, reagents and methods would be also useful, particularly for the most isolated laboratories where immediate help is not available.

The challenge in implementing effective internal quality control is to ensure a “culture of quality” in peripheral laboratories so that staff understand the concepts and needs for quality control and assurance.
## Annex 1

### Model List of Supplies and Equipment for a Malaria Microscopy Laboratory in a Small Clinic Setting

<table>
<thead>
<tr>
<th>ITEM</th>
<th>QUANTITY</th>
<th>TYPICAL PACKAGING</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CONSUMABLES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLOVES, examination, latex,</td>
<td>15 boxes</td>
<td>50 pairs/box</td>
<td>Approx. 3 months supply/person</td>
</tr>
<tr>
<td>disposable, large</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLOVES, examination, latex,</td>
<td>15 boxes</td>
<td>50 pairs/box</td>
<td>Approx. 3 months supply/person</td>
</tr>
<tr>
<td>disposable, medium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLOVES, examination, latex,</td>
<td>6 boxes</td>
<td>50 pairs/box</td>
<td>Approx. 3 months supply/person</td>
</tr>
<tr>
<td>disposable, small</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LENS CLEANING SOLUTION</td>
<td>1</td>
<td>1 litre bottle</td>
<td></td>
</tr>
<tr>
<td>MARKER, black, dye/bleach/wafer</td>
<td>6</td>
<td>Roll</td>
<td></td>
</tr>
<tr>
<td>resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COTTON WOOL, hydrophilic</td>
<td>1 Roll (500 grams)</td>
<td>500 cotton swabs</td>
<td>Approx. 3 months supply</td>
</tr>
<tr>
<td>LANCET, disposable, sterile,</td>
<td>10 boxes</td>
<td>200 lancets/box</td>
<td>Approx. 3 months supply</td>
</tr>
<tr>
<td>standard type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHARPS CONTAINER, needles and</td>
<td>10</td>
<td>individual</td>
<td>Approx. 3 months supply</td>
</tr>
<tr>
<td>syringes, 15 l., cardboard for</td>
<td></td>
<td>packaging</td>
<td></td>
</tr>
<tr>
<td>incineration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEEDLE, sterile, 21 grams</td>
<td>1 box</td>
<td>100 needles/box</td>
<td>Approx. 3 months supply</td>
</tr>
<tr>
<td>TUBE, VACUUM, EDTA, 3 ml., purple</td>
<td>1 box</td>
<td>100 tubes/box</td>
<td>Approx. 3 months supply</td>
</tr>
<tr>
<td>TUBE, VACUUM, PLAIN, 3 ml., red</td>
<td>1 box</td>
<td>100 tubes/box</td>
<td>Approx. 3 months supply</td>
</tr>
<tr>
<td>SYRINGE, disposable, luer, 5 ml.</td>
<td></td>
<td>100 syringes</td>
<td>Approx. 3 months supply</td>
</tr>
<tr>
<td>SAMPLING SET, with wings, 23</td>
<td>1 pack</td>
<td>200 pieces/pack</td>
<td>Aprox. 6 months supply</td>
</tr>
<tr>
<td>grams</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLIDE, 76 mm x 26 mm, 1.0 mm</td>
<td>60 boxes</td>
<td>50 slides/box</td>
<td></td>
</tr>
<tr>
<td>– 1.2 mm thickness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FILTER PAPER suitable for PCR</td>
<td>2 packs</td>
<td>100 discs/pack</td>
<td></td>
</tr>
<tr>
<td>sampling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LENS CLEANING PAPER, sheet</td>
<td>1</td>
<td>100 sheets/booklet</td>
<td></td>
</tr>
<tr>
<td>PIPETTE, TRANSFER (Pasteur),</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>graduated, plastic, non-sterile</td>
<td>500 pipettes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DETERGENT</td>
<td>5 litres</td>
<td></td>
<td>Local purchase</td>
</tr>
<tr>
<td>ITEM</td>
<td>QUANTITY</td>
<td>TYPICAL PACKAGING</td>
<td>COMMENTS</td>
</tr>
<tr>
<td>---------------------------------------------------------------------</td>
<td>----------</td>
<td>-------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>CHLORINE, 1g (NaDCC/ Sodium dichloroisocyanurate 1.67g) tablets</td>
<td>100</td>
<td>100</td>
<td>1 tablet provides 0.2 l. of a 0.5% chlorine solution. 100 tablets = 10 l. of a 1% solution/l.</td>
</tr>
<tr>
<td>PENCIL, grease, red glass writing</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NON-CONSUMMABLES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FILTER, water, 10 l., fountain, 4 candles</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(water filter, 10 l.) CANDLE + COLLAR + GASKET</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOTTLE, swan neck jet, plastic, 250 ml.</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FUNNEL, plastic, 90 mm diameter, short end</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RACK, for slides, expandable, stainless steel</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMER, digital, 60 mn with alarm</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RACK FOR DRYING SLIDES, vertical, plastic, holds 10 slides</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYLINDER, MEASURING, plastic, graduated, spout, 100 ml.</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYLINDER, MEASURING, plastic, graduated, spout, 250 ml.</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FUNNEL, glass, 100 mm diameter, short end</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPATULA, double, for analysis, stainless steel</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOTTLE, glass, brown, screw cap, 1 l.</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TALLY COUNTER, 4 digits, hand operated</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MICROSCOPE LIGHT</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MICROSCOPE LIGHT, battery-powered e.g. with white LED light</td>
<td>1</td>
<td></td>
<td>If no reliable external power source</td>
</tr>
<tr>
<td>SLIDE BOX, for 100 slides</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUOJ BOTTLES, 5 ml.</td>
<td>300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEAKER, graduated, glass, 100 ml.</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEAKER, graduated, plastic, 100 ml.</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEAKER, glass, Pyrex 500 ml.</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAINING JAR, glass, with lid</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROD, glass, 250 mm diameter 6mm–7mm</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Annex 1: Supplies and Equipment for Small Clinic

<table>
<thead>
<tr>
<th>ITEM</th>
<th>QUANTITY</th>
<th>TYPICAL PACKAGING</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLASS OR POLYETHYLENE STAINING TROUGHS with lids (e.g. for 20 double thickness slides)</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPREADER (for making blood films)</td>
<td>2</td>
<td></td>
<td>optional</td>
</tr>
<tr>
<td><strong>EQUIPMENT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MICROSCOPE, binocular with electric light source, x10 (and x7) eyepieces and oil immersion lens</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH meter</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIPETTE, calibration KIT</td>
<td>1</td>
<td>30 tests</td>
<td>If not otherwise available</td>
</tr>
<tr>
<td>PIPETTE, adjustable volume, 100 µL–1000 µL</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>REAGENTS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IODINE Povidone, 10% solution</td>
<td>3</td>
<td>200 ml., dropper bottle</td>
<td>Approx. 3 months supply</td>
</tr>
<tr>
<td>OIL, IMMERSION</td>
<td>1</td>
<td>500 ml., bottle</td>
<td>Enough for approx. 10000 slides when using 50 µl of oil (drop)</td>
</tr>
<tr>
<td>ISOPROPYL ALCOHOL</td>
<td>1</td>
<td>1 l., bottle</td>
<td></td>
</tr>
<tr>
<td>METHANOL</td>
<td>2</td>
<td>1 l., bottle</td>
<td>Approx. 2000 slides can be fixed with 1 l. methanol</td>
</tr>
<tr>
<td>pH calibration solution, pH 4.0, TPL 250 ml.</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH calibration solution, pH 7.0, TPL 250 ml.</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH calibration solution, pH 10.0, TPL 250 ml.</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLEANING SOLUTION (pH meter) general purpose</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>POTASSIUM DIHYDROPHOSPHATE, 500 grams</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DISODIUM HYDROGEN PHOSPHATE, 500 grams</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUFFER, pH 7.2, dose for 1 l.</td>
<td>100 tablets</td>
<td>Tablet for 1 l. (100 tablets)</td>
<td></td>
</tr>
<tr>
<td>GIEMSA STAIN</td>
<td>10</td>
<td>500 ml. bottles</td>
<td></td>
</tr>
<tr>
<td><strong>Manuals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMCP Standard Operating Procedures for malaria microscopy</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Annex 2

Model List of Supplies and Equipment for Accreditation of Expert Microscopists

1. Teaching equipment

For teaching sessions and group discussions, the following items should be ideally available:

- overhead projector;
- screen for slide projection (a white sheet is an adequate substitute, but a whiteboard is unsuitable because it will reflect projected light);
- flipcharts, one for each small group of learners, and supplies of butcher’s paper or newsprint are usually cheap and readily available;
- whiteboard;
- marker pens for whiteboard markers (blue, red and black);
- permanent markers (blue, red and black); and
- electronic timer (for practical slide examination timing).

2. Trainees equipment

The equipment listed below should be provided for each learner. Where supplies have to be ordered, this should be done well in advance of the course. Many items are difficult to obtain at short notice.

- Microscope: try to determine the type of microscope that trainees will use when they return to their places of work. There is less value in training them to use microscopes that use artificial light if they will later have to work with natural light. It may be possible for trainees to bring the microscopes that they will use in their work, but it is preferable to provide microscopes. It is stressed, however, that all malaria microscopy programmes should now be moving to the use of artificial light sources only.
- Notebook. This should be used only for occasional notes or instructions as availability of the Learner’s Guide should eliminate the need for notes to be routinely taken during training sessions.
- Black ballpoint pen.
- Set of pencils (medium-hard graphite, plus red, blue, brown and black) for drawing diagrams of suspected parasites during practical sessions.
- Pencil sharpener.
- Eraser.
- Ruler.
Annex 2: Supplies and Equipment for Accreditation

- Tally counter x 2.
- Calculator.
- Set of reference slides with Giemsa-stained thick and thin blood films, containing the species of malaria parasite each trainee is most likely to see in routine work. Normally, the species would be *Plasmodium falciparum*, *P. vivax* and *P. malariae*, although *P. ovale* is being seen increasingly, both in Africa and in the rest of the world. Unfortunately, good specimens of *P. malariae* and *P. ovale* are difficult to obtain. Note that, under normal tropical conditions, these reference slides will have a shelf-life of only about six months.
- Set of about 25–30 Giemsa-stained thick and thin blood films for use in practice sessions. These should show the species of malaria parasite common in the area where trainees will work, at different stages and in various densities. If a national slide bank is unavailable, collecting these slides will require a lot of time and effort, but is ultimately worthwhile since practical sessions will proceed much more smoothly if facilitators know exactly what each learner is examining.

### 3. Laboratory equipment

<table>
<thead>
<tr>
<th><strong>Optical equipment</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscope binocular</td>
<td>1 per trainee</td>
</tr>
<tr>
<td>Blue (opaque) filters for the sub-stage microscope lamp</td>
<td>1 per trainee</td>
</tr>
<tr>
<td>Objective markers (diamond-pointed) for “ringing” interesting parasites and specimens</td>
<td>1 per group of 4–5 trainees</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Other equipment/consumables</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Boxes of paper tissues</td>
<td>1 per group of 4–5 trainees</td>
</tr>
<tr>
<td>Lens tissue</td>
<td></td>
</tr>
<tr>
<td>Immersion oil</td>
<td></td>
</tr>
<tr>
<td>Wooden boxes for microscope slides, with hinged lids, for 100 slides stored vertically</td>
<td>1 per trainee plus 6 spares</td>
</tr>
<tr>
<td>Wooden boxes for microscope slides, with hinged lids and carrying handles, for 50 slides stored horizontally (sometimes called field collection boxes or WHO slide boxes)</td>
<td>2 per group of 4–5 trainees</td>
</tr>
<tr>
<td>Record forms</td>
<td>Stocks as appropriate</td>
</tr>
</tbody>
</table>
Annex 3

Model Theory Test for Accreditation of Expert Malaria Microscopists

1. What is the most important thing you should ensure before taking a blood sample from a patient?
2. The erythrocytic cycle of \textit{P. falciparum} has a duration of how many hours?
3. What are malaria parasite sexual forms called?
4. While in the mosquito’s stomach, the microgametes penetrate the macrogametes and generate what?
5. What are the dormant stages of \textit{P. vivax} and \textit{P. ovale} called?
6. In which part of the world is \textit{P. ovale} mainly found?
7. What is the best pH for the buffer used in preparing Giemsa malaria stain?
8. Why is it not good to have thick and thin smears on one slide?
9. Describe how you can check a thick smear to make sure it is the correct thickness.
10. List one common artefact seen in blood films.
11. What are Howell-Jolly bodies?
12. State one important action to do with your microscope when you have finished using it.
13. Describe the appearance of mature trophozoites seen in \textit{P. vivax}.

14. What is the characteristic (but not always seen) shape of the red blood cells (RBC) in \textit{P. ovale}?
15. Which human malaria parasite species can have multiple infections in red blood cells?
16. How many merozoites are usually seen in \textit{P. vivax}?
17. How many merozoites are usually seen in \textit{P. ovale}?
18. How many merozoites are usually seen in \textit{P. falciparum}?
19. Schuffner’s dots are seen in which human malaria species?
20. What human malaria species may contain Ziemann’s stippling?
21. What human malaria species may contain Maurer’s clefts?
22. Before calling a malaria slide negative, what is the lowest number of high power fields that should be examined?
23. If the patient’s white blood cell count (WBC) is not known, what value does WHO recommend using in parasite count calculations?
24. If 50 parasites are seen when 200 white blood cell are counted, what is the parasite count per µl (in this example the true white blood cell count is 5000/µl)?
25. Give one advantage and one disadvantage of rapid diagnostic tests (RDTs) for malaria diagnosis.

\textbf{SCORE:} \hspace{1cm} /25
Annex 4
Checklist and Reporting Form for Supervisory Visits to Malaria Microscopy Laboratories

The consultative visits should ensure that these issues are addressed.

Organizational issues:

- written standard operating procedures are available;
- an adequate supply of reagents within expiration dates is available;
- well-maintained microscopes of high quality are available;
- there is a satisfactory supply of quality reagents and consumables;
- internal quality control is performed at the required intervals;
- laboratory safety practices are observed;
- record-keeping is accurate and consistent with requirements;
- results are promptly reported to treatment centre or physicians;
- patient slides are available and properly stored for cross-checking either during consultative visits or by submission to the intermediate level;
- staff have received adequate training with refresher courses or corrective action recommended when appropriate; and
- the workload is monitored and is satisfactory.

Technical issues:

- blood slide collection procedures are properly carried out;
- procedures for smear preparation, staining and examination are in accordance with National Malaria Control Program (NMCP) standards;
- control slides that are known to be positive and negative should be 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; and
- any significant problems requiring strategies and systems for improvement are documented.
Supervisory Visit Report Form for Assessment of Malaria Microscopy Laboratories

I. General Information

<table>
<thead>
<tr>
<th>Name of laboratory/facility:</th>
<th>Date of visit (mm/dd/yyyy)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Type of facility:

- [ ] Rural Clinic
- [ ] District Hospital
- [ ] Provincial Hospital
- [ ] Private Hospital
- [ ] Other

Address of the laboratory:

__________________________________________________________________________
Municipality: __________________________ Province: ______________________
Telephone/Fax: __________________________ E-mail: _________________________
Name of head of laboratory: __________________________
Name of facility head/director: __________________________
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

II. Procedures

A. Archiving of slides

- Average number of slides read/month
- No. of slides read/day*
- No. of slides archived/assessed by validator

(*) For highly endemic areas

B. Blood film preparation

- No. of smears reviewed

- Thick and thin smear prepared?
- Staining technique used
  a. Giemsa
  b. Other (specify)

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
<th>Good</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>___</td>
<td>___</td>
<td>____</td>
<td>____</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
<th>Good</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>___</td>
<td>___</td>
<td>____</td>
<td>____</td>
</tr>
</tbody>
</table>
### C. Performance (review of slides), cross-checking or validation

<table>
<thead>
<tr>
<th>Detailed Item</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of slides cross-checked by validator</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agreement (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>False-positives (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>False negatives (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thick films too thin (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thick films too thick (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slides poorly stained (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slides containing stain precipitate (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference slides provided by the validator and read by the laboratory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of slides read</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agreement (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>False-positives (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>False negatives (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### III. Laboratory set-up

<table>
<thead>
<tr>
<th>Detailed Item</th>
<th>Good</th>
<th>Poor</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bench space</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sink/washing area/staining area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Access to clean water supply</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural lighting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Power source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventilation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Storage space for supplies and materials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waste management system</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### IV. Microscopes

<table>
<thead>
<tr>
<th>Detailed Item</th>
<th>Yes</th>
<th>No</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>The microscope(s) is binocular.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>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.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood films can be brought into sharp focus at x100 oil immersion magnification.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The stage movement mechanism is precise and stable.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The microscope(s) is regularly serviced.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The microscope(s) is protected with a cover when not in use.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylene is never used to clean the microscope(s), objectives or eye pieces.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spare bulbs are stored in the laboratory.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## V. Microscope slides

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscope slides are of good quality and are thoroughly cleaned before use.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscope slides do not have scratches or surface aberrations.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscope slides do not give a blue background colour after staining.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscope slides do not have fungal contamination.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscope slides that have been damaged by fungus are discarded and not used again.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In programmes with high humidity, microscope slides are protected against fungal contamination.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## VI. Stain solutions

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock staining solutions are stored in the dark and far from a heat source.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commercial stain solutions are within the manufacturer’s expiry date.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commercial stain solutions do not contain excessive stain precipitate.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The cap of the reagent bottle is always tightly sealed except when stain is being removed for use.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stain is always removed from the reagent bottle using a clean pipette or something similar.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water is never added to the stock stain solution.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unused stain is never returned to the stock bottle.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## VII. General laboratory supplies

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol and cotton (or similar) for cleaning skin prior to blood collection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lancets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffer salts or buffer tablets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH meter that reads to two decimal places</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH calibration solutions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staining jar</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Annex 4: Checklist for Supervisory Visits

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staining rack</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drying rack</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graduated cylinders of the correct size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash bottles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Timers – sufficient number for staining and for each microscopist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immersion oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tally counters – sufficient number for the number of staff</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lens paper</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slide boxes for storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For laboratories that prepare stain from powder – sufficient glycerol, methanol, powder, beakers, measuring cylinders, filter paper, funnels, stirring rods, scales, spatulas and storage bottles</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### VIII. Documentation

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Logbook or record book located in the laboratory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient’s name and details recorded in an organized and legible manner</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date smear collected/received recorded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species identification performed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parasite counting performed (if yes, specify method used under remarks column)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Forms**

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathology request forms used</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Result/report forms completed correctly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Referral forms used</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Analysis**

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monthly analysis prepared including the number of slides examined, positivity rate, percentage of species identified (as appropriate) and quantitation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Stain Quality Control Register

There is a Stain QC Register recording the brand, batch numbers, quantity, date of receipt and the date of expiry (if applicable) of all stain powders or solutions, methanol and glycerol used by the laboratory. For stains prepared from powders - the preparation date each batch of stock stain is prepared. For all stains - the staining time and pH for each new batch, and any problems encountered.

### Reference Materials

- SOPs located in the laboratory
- Technical Manual and Bench Aids located in the laboratory
- Operating and Maintenance Manuals available (if appropriate)
- Microscope maintenance logbook available

### IX Biosafety

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Laboratory staff wear protective laboratory coats/gowns
- Staff wear gloves when collecting blood samples
- Hand-washing facilities with soap (or similar) available
- The power supply for the microscope(s) and laboratory lighting in good condition and safe

### Proper disposal units

- 1. Containers for dry waste
- 2. Containers for infectious materials
- 3. Puncture resistant container for sharps

### X. Quality and performance indicators

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- The laboratory fully complies with the national QC protocol.
- There is a formal protocol available for the analysis of QC results and for taking corrective action if the results are not satisfactory.
- The laboratory performance in the national QC protocol is satisfactory.
Annex 4: Checklist for Supervisory Visits

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The laboratory has an additional internal QC programme for monitoring the quality of slide preparation and staining.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The laboratory measures workload.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>There is a protocol to manage workload during periods of staff absences (sickness, holidays, etc.).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**XI. Drug dispensing**

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The laboratory dispenses drugs to positive cases?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>How?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Why?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**VIII. General findings and recommendations**

____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________

**IX. Supervisor/Validator’s comments:**

____________________________________________________________________
____________________________________________________________________
____________________________________________________________________

**Accomplished by:**

Name of Validator: ____________________________________________________
Signature of Validator: ________________________________________________
Date: _________________________________________________________________
Annex 5

The Rationale and Principles of the Recommended Method for the Selection of Slides for Validation

Rationale

Two methods were recently evaluated in an attempt to develop a simple and cost-effective system for the selection of malaria slides for cross-checking. These were a modified small lot QC method similar to the Lot Quality Assurance System (LQAS) used for tuberculosis QC, and a method based on the monthly selection of five low-density and five negative slides.

The modified LQAS method is based on laboratories not exceeding a fixed number of errors (the number of acceptable errors is calculated on the prevalence and on the power of statistical analysis required). Test centres pass or fail depending upon whether they are below or above the stipulated number of acceptable errors. The methodology has the advantage that it allows a small number of slides (100–120 per year) to be selected for cross-checking with a sound statistical basis for interpreting the results. However, the method is cumbersome and difficult to use for malaria quality assurance for several reasons. The selection of the sample size is relatively complicated and not necessarily clear to all laboratory staff since it is based on prevalence, the number of slides examined and the number of acceptable errors. These factors are problematic for malaria microscopy because of the seasonal variation in prevalence. This contrasts with tuberculosis microscopy where LQAS has been endorsed as an appropriate method of quality control. In addition, while the method reliably detects poor performers, it does not differentiate between moderate and good performers. The random selection of slides also means that strong positives will be included in the quality control sample which may reduce the analytical value of the method.

The proposed QC protocol in Chapter 9, based on the monthly selection of five low-density and five negative slides, primarily analyses results in the form of percentage agreement, which is influenced by both false-positive and false negative results. Therefore, it is an estimation of the overall performance of a laboratory or test centre.

\[
\text{Percentage Agreement} = \frac{\text{number of correct QC results}}{\text{number of all QC results}} \times 100
\]
Annex 5: Selection of Slides for Validation

As the QC protocol uses a biased sample of weak positive slides rather than all positives slides, and as weak positive slides are likely to have a higher false-positivity rate than strong positive slides, the percentage agreement may underestimate the actual routine performance of a laboratory or test centre in routine testing.

The true false-positive rate, sensitivity and specificity cannot be directly calculated from the QC data because the positive/negative distribution of the QC sample is not the same as the positive/negative distribution of routine slides examined by the laboratory or test centre. To calculate the true false-positive rate, it is necessary to also know the proportion of weak positive results in routine testing.

Example:

Over a four-month period, a laboratory reports 500 positive blood films, including 450 strong positive and 50 weak positive results. During this same period, 20 weak positive thick films are randomly selected for cross-checking (five weak positives each month) and two of these blood films are found to be negative.

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

2. Assumptions and limitations

The QC protocol assumes that all QC samples are selected in a random manner strictly according to the selection criteria detailed in the protocol, and are cross-checked with a high degree of accuracy in a blinded and unbiased manner.

It also uses a minimum sample size to achieve maximum practicality and sustainability in field conditions. An accepted limitation of this approach is that the reduced sample size may reduce the statistical stringency of the analysis of results. This is in part compensated for by the use of four-month cohort analysis, selecting (for microscopy) an equal number of weak positive and negative slides. It is also based on the premise that it is better to perform less QC well, than more QC poorly.

The protocol cannot be used for laboratories that report only positive and negative microscopy results and do not grade results. Such laboratories require significantly larger QC sample sizes to compensate for the limited analytical value of cross-checking strong positive slides.

21 This is because the QC sample requires an equal number of positive and negative slides.
As the protocol stipulates that only weak positive slides must be cross-checked, the QC results may have a higher false-positive rate than the actual routine performance of the laboratory.

When cross-checking is not performed by a reference laboratory, the expertise of the cross-checking may be reduced. This approach to QC is used out of necessity rather than preference.

As the protocol requires five weak positive and five negative slides to be cross-checked each month, it is possible that the cross-checker may anticipate the result of some of the slides. For example, if the cross-checker has identified five weak positive slides, the cross-checker may assume that the remaining slides are negative. It has to be emphasized to cross-checkers that, due to the possibility of false-positive and false-negative results, the QC sample does not necessarily contain an equal number of true positive and negative slides.

It is accepted that for very weak positive microscopy slides the cross-checker may report a false negative result. This is a natural limitation of microscopy examination. The statistical probability of a cross-checker failing to detect weak positivity has been calculated as follows:

<table>
<thead>
<tr>
<th>True number of parasites per 100 fields</th>
<th>Probability of a false negative result examining:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 fields</td>
</tr>
<tr>
<td>1</td>
<td>34.8%</td>
</tr>
<tr>
<td>2</td>
<td>12.1%</td>
</tr>
<tr>
<td>3</td>
<td>4.2%</td>
</tr>
<tr>
<td>4</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

### Annex 6

**Model Report Form for Cross-checking Malaria Blood Slides**

**MONTHLY RETURN – NO SPECIES IDENTIFICATION**

<table>
<thead>
<tr>
<th>Validation Centre:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory Name:</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Month</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>February</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>March</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>June</td>
<td></td>
<td></td>
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<tr>
<td>July</td>
<td></td>
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<tr>
<td>August</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>September</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>October</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>November</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>December</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Observer 1-laboratory**

<table>
<thead>
<tr>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>C</td>
<td>D</td>
</tr>
</tbody>
</table>

**Observer 2 - cross-checker**

<table>
<thead>
<tr>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>C</td>
<td>D</td>
</tr>
</tbody>
</table>

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

Enter the values for A, B, C, D in the 2x2 table for each month. Send the completed reports to the QC supervisor.

**Comments on quality of blood film and staining smear:**

97
Annex 7

Model Report Form for Cross-checking Malaria Blood Slides

MONTHLY RETURN – SPECIES IDENTIFICATION

<table>
<thead>
<tr>
<th>Validation Centre:</th>
<th>Laboratory Name:</th>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Month</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
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<td>February</td>
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<tr>
<td>December</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Observer 2 - cross-checker</th>
<th>Observer 1 - laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. falciparum NOT present</td>
<td>A</td>
</tr>
<tr>
<td>P. falciparum NOT present</td>
<td>B</td>
</tr>
<tr>
<td>P. falciparum NOT present</td>
<td>C</td>
</tr>
<tr>
<td>P. falciparum NOT present</td>
<td>D</td>
</tr>
</tbody>
</table>

A = number of slides reported as containing *P. falciparum* (either as a single or mixed infection) by both readers
B = number of slides reported as containing *P. falciparum* only in routine testing by the laboratory but the presence of *P. falciparum* was not confirmed by the cross-checker (incorrect species identification)
C = number of slides reported as *P. falciparum* not present in routine testing by the laboratory but found to be present by the cross-checker either as a single or mixed infection (incorrect species identification)
D = number of slides reported as not containing *P. falciparum* by both readers

Enter the values for A, B, C, D in the 2x2 table for each month. Send the completed reports to the QC supervisor.

Comments on quality of blood film and staining smear:
Annex 8

Standard Operating Procedures for Establishing a National Reference Slide Bank

The standard operating procedures below describe the procedure of selection, collection, preparation, staining and further processing of the specimens required for a national slide bank. The SOPs for cleaning slides and staining blood films can be found in the WHO training manuals: Basic Malaria Microscopy, Part I. Learner’s Guide and Basic Malaria Microscopy, Part II. Tutor’s Guide.

1. Technical responsibility

The senior laboratory technologist has the responsibility to ensure that the slide bank process is carried out according to the SOPs.

Each slide bank team member must be able to:

- describe the objectives of the slide set,
- list their individual responsibilities,
- demonstrate the ability to carry out designated tasks,
- describe back-up plans to anticipated events, and
- demonstrate teamwork and the ability to repeatedly provide materials to standards of excellence.

2. Pre-implementation requirements for collection of samples

Collection of samples in the field can be carried when the following activities have been completed:

- establishment of a national slide bank has been approved;
- funding is available;
- staff selected and trained, or their abilities confirmed;
- the specimens that are required have been listed and selection methods agreed; and
- slide cleaning and wrapping, buffer solutions, stain stock solutions and other preparatory measures have been completed.
3. Steps carried out before departure to the field

3.1 Cleaning and storing micro-slides for slide bank activities

REMEMBER: Poorly cleaned slides can lead to substandard blood films and to imprecise microscopy and diagnosis and can affect the quality and reliability of slide bank materials. Ensure slides are properly cleaned, wrapped and stored.

Slide bank activities can use large numbers of slides that must be cleaned, wrapped and stored before fieldwork starts.

3.1.1 Equipment and materials

- New “superior” quality micro-slides, with ground edges and a frosted end for labelling. (Allow 200+ each when thin and thick films are made on separate slides).
  **Do not use recycled slides for slide bank activities.**
- Two medium-sized plastic bowls or basins.
- Detergent liquid.
- Washing cloths or soft sponges (as required).
- A supply of clean, lint-free cotton cloths (the domestic kind used to dry glassware).
- A good supply of clean water.
- Sheets of clean paper cut to 11 cm x 15 cm.
- Empty slide boxes of the type in which new slides are supplied.
- Clear adhesive tape.
- Desiccators and activated silica gel.
- A locally made warm cupboard, i.e. a cupboard with tight-fitting doors and one 25 Watt bulb per shelf.

3.1.2 Method

Cleaning is best carried out as a small group activity as follows.

(1) Separate and place new slides in a warm detergent solution for 2–3 hours to soak.
(2) Use the washing cloth or sponge to clean each slide on both sides by rubbing the two surfaces between the forefinger and thumb.
(3) Individually rinse slides in clean water to remove all traces of detergent.
(4) Handle slides by the edges, to drain excess water from each slide before drying it using a clean, cotton cloth.
(5) Discard any chipped or scratched slides.
(6) Using the cut paper, wrap the dried slides in packs of 10. The ends of the wrapper can be folded and secured with adhesive tape. Place them in the cardboard boxes ready for use with the box secured with a rubber band.
(7) Each box normally holds 10 packs of wrapped slides so the calculation of the number of slides available for use is simple.
(8) Store boxed clean slides in a warm cupboard or desiccator to ensure they remain completely dry until required.
(9) For quality control (QC), label each box with details of the cleaning date and the name of the person(s) responsible for cleaning and wrapping.

**REMEMBER:** In warm, humid climates, fungal growths are quickly established on glass surfaces. Cleaned, wrapped slides must be stored in a warm cupboard or in a desiccator with active silica gel.

### 3.1.3 Making up a stock solution of Giemsa stain

Giemsa stain is commercially available as a ready-to-use solution, or can be made up by the NMCP or laboratory services to ensure quality and distributed throughout the laboratory and malaria control programme network. If supplies are unavailable, Giemsa can be made-up as follows:

**Formula**

- Giemsa powder, 3.8 g
- Methanol, 250 ml.
- Glycerol, 250 ml.
- Solid glass beads, 5 mm diameter: use about 50
- One screw-topped, large, dark glass bottle, clean and dry

**Preparation**

1. Ensure bottle is clean and dry. Place the beads into the bottle.
2. Pour in the methanol.
3. Add stain powder.
4. Tightly close the bottle and shake it in a circular motion for 2–3 minutes.
5. Add the glycerol and repeat the shaking.
6. Continue shaking for 2–3 minutes, at least six times in the day.
7. Repeat the shaking every day for 2–3 days until the stain is thoroughly mixed.
8. Label the bottle and store in a cool place away from direct sunlight.
9. If using a clear glass bottle, cover with a thick paper jacket to avoid light penetration.

With stock solution, it is important to remember:

- The lid should be kept tightly screwed to prevent evaporation of the solvent and oxidation of the stain.
- For daily use, small amounts of the settled stain should be decanted into a 25 ml. bottle. Avoid contaminating the stock solution with water.
- Water **should not** be added to the stock solution; the smallest amount causes the stain to deteriorate, making staining progressively ineffective.
The bottle **should not** be shaken before use. Shaking re-suspends precipitates which settle on blood films during staining and obscure important details during microscopy.

Unused stain **should not** be returned to a stock bottle or to the bottle used in the daily routine; stain out of the bottle must be used quickly or discarded.

One batch of stain **should not** be made up for use throughout the day. Giemsa stain once diluted rapidly loses its staining properties so slides will stain very poorly by the end of the day if this method is used.

### 3.1.4 Buffered water

To correctly stain blood films, water buffered to pH 7.2 must be used to dilute the Giemsa stain.

**Note on pH and Giemsa staining**

Giemsa stock stain should normally be prepared by diluting the stain with water that has been buffered to pH 7.2. This will achieve optimal staining quality. Some available Giemsa preparations require the pH to be varied to optimize the quality of staining. In such cases, it is recommended that the national reference laboratory determines the optimal pH and preparation conditions to achieve this, and adjusts SOPs for slide preparation for other laboratories using this product, accordingly. It follows from this that Giemsa stock solution procured for a programme should be standardized to minimize the requirement for adjustment of staining SOPs.

Buffer salts should be weighed using an analytical balance. Ensure that buffer salts are stored correctly and cannot absorb moisture from the air.

Formulated buffer tablets are available to give a specific pH when mixed with a fixed amount of water. These are useful where facilities are limited but must be kept in an airtight container to avoid absorbing moisture.

**To make up pH 7.2 buffered water**

**Equipment and supplies:**

- Analytical balance, weighing to 0.01 g
- Filter papers, 11 cm in diameter
- One glass conical flask, 1 l. capacity
- One glass beaker, 250 ml. capacity
- Wooden spatulas (wood tongue depressors are usually available)
- Distilled or de-ionized water, 1 l.
- Potassium dihydrogen phosphate (anhydrous) (KH$_2$PO$_4$)
- Disodium hydrogen phosphate (anhydrous) (Na$_2$HPO$_4$)
Procedure:

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

The buffer water is now ready for adjusting to pH 7.2 with the correcting fluid.

Making up the 2% correcting fluids

Equipment and supplies:

- An analytical balance readable to 0.01 g
- Filter papers, 11 cm in diameter
- Two glass bottles with stoppers each of 100 or 150 ml. capacity
- Potassium dihydrogen phosphate (anhydrous) (KH$_2$PO$_4$)
- Disodium hydrogen phosphate (anhydrous) (Na$_2$HPO$_4$)
- Distilled or de-ionized water, about 200 ml.
- Wooden spatulas
- Two beakers of 250 ml. capacity
- One measuring cylinder of 100 ml. capacity
- Labels

Procedure:

1. Follow steps 1 and 2 of the method for making buffered water, then move the gram weight a further 2 g along the scale arm.
2. Weigh 2 g of disodium hydrogen phosphate and add it to 100 ml. of water in the beaker; stir with the spatula until the salts have dissolved.
3. Pour the solution into one of the glass bottles and label the bottle “2% disodium hydrogen phosphate”.

103
(4) Repeat steps 1 to 3, only this time using 2 g of potassium dihydrogen phosphate and label the bottle.

Bottles should be stored in a cool place away from sunlight.

3.1.5 Checking and adjusting the pH of buffered water

Buffered water should be checked for pH at the beginning of the working day. To adjust the pH, add small quantities of the correcting fluids to the buffered water – 2% Na$_2$HPO$_4$ if the pH is below 7.2 (too acid), or 2% KH$_2$PO$_4$ if the pH is above 7.2 (too alkaline). Adjustment follows the method outlined below:

REMEMBER: Some programmes may use other systems to measure pH. There are many reliable kinds of pH meter in use.

Equipment and supplies:

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

Procedure:

(1) Follow instructions provided with the pH testing kit.
(2) Adjust the pH of the water in the conical flask by adding small quantities of the appropriate correcting fluid.

4. The overall process

The following steps emphasize the intricacy of the routine. The medical technician responsible for the slide set activities should ensure that support staff have the necessary skills to:

(1) Select a potential donor

(a) Take blood by finger-prick.
(b) Make a thick and thin blood film on the same slide or on separate slides.
(c) Stain the films with Giemsa stain.
(d) Use thick blood film microscopy to determine the presence and density of malaria parasites.
(e) When parasites are present, name the stages and species—the thin film may be used to confirm the diagnosis.
(f) Decide specimen suitability for inclusion in the slide set—including confirmation.
(2) Obtain an additional specimen from a donor

- (g) Inclusion in the slide bank may require visiting the patient at home.
- (h) Ensure the potential donor meets all criteria outlined by NMCP.
- (i) Inform patient, or parent/guardian, of reasons for requesting specimen.
- (j) Obtain signed consent, ensuring all ethical aspects are followed.
- (k) Bring patient (and guardian) back to operations centre, if appropriate.
- (l) Collect further specimen by finger-prick to confirm it meets requirements.
- (m) Double-check the diagnosis and patient details, while making the donor comfortable.

(3) Following confirmation of diagnosis and inclusion in the programme

- (n) Put 3 ml.–5 ml. of blood into an EDTA-treated container by venepuncture.
- (o) Ensure donor is returned or able to return home.
- (p) Take a suitable aliquot of blood (mix well) or make a blood spot on filter paper (e.g. Whatman 3m) for subsequent PCR confirmation of diagnosis if PCR is available.
- (q) Make 200 thick and 200 thin blood films from the specimen.
- (r) After drying the films (and fixing thin films) stain in Giemsa.
- (s) Rinse and dry films.
- (t) Confirm microscopically, the diagnosis, density and quality of films and staining (link with later PCR result, when available).
- (u) Temporarily label slides awaiting PCR result.
- (v) Pack and store slides for transportation back to slide bank and future use.
- (w) When PCR result available, finalize coded labels and registration.

4.1 The selection of material suitable for the slide bank.

The arrangements for screening potential donors may be influenced by factors such as the location of communities and their cultural or religious beliefs.

4.1.1 Screening patients by blood film examination

Record patient details in the register (see Annex 14 and 15 for model reporting forms) and make a thick and thin blood film, on the same slide following the established methods. A unique identifier (site code and study number) should be assigned to each sample later to avoid traceability to the individual patient or subject. The “study number” should be the number of the study subject assigned sequentially as subjects are enrolled.

The thick film should be is used to search for malaria parasites and for establishing the stages and species present. The thin film may be used to confirm the species diagnosis.

23 Steps (g) and (k) are seldom required. If the time lapse between definite diagnosis and availability of the donor is short, then only steps (h) and (m) need be followed.

24 Step (m) is not necessary if the sample can be taken soon after initial identification.
Contamination with blood is a potential risk to worker and patient. Use the following precautions:

- Wear protective gloves when handling blood and remove them before leaving the work area or writing notes.
- Avoid getting blood, wet or dry, on fingers and hands.
- Cover cuts with a waterproof dressing.
- Avoid pricking oneself with a contaminated sharp instrument.
- Use lancets and syringe needles only once, disposing them after use in a sharps container.
- After finishing a task, wash hands with soap and water.
- Quickly wipe blood off skin with alcohol-damp cotton wool.
- Delineate “dirty” bench areas for slide preparation and clean areas for record management and keep these areas strictly separate.

**Equipment and supplies:**

- Latex protective gloves (powder free), three pairs per worker
- Cleaned and wrapped slides, with a frosted end for use as a label
- Paper sheets with printed templates for blood films
- Sterile lancets, one per person, plus 10%
- Sharps container
- 70% ethanol
- Absorbent cotton wool
- Slide box or tray and cover to dry slides horizontally protected from dust and flies
- 4–6 clean, lint-free cotton cloths for drying slides
- Record forms and/or register and pen

In most countries, regulations govern the disposal of contaminated lancets, needles, broken slides and other materials. They are best discarded into a commercially available, yellow “sharps container” that can later be correctly discarded. In the absence of a sharps container, a bottle with a narrow mouth can be used and later incinerated. Never fill a sharps container to the rim. Waste should not be left at the peripheral facilities since they usually have poor disposal facilities. Carry the sharps container in strong plastic bags/boxes for central disposal.

### 4.1.2 Staining blood films

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

This method is used to stain a few slides for a quick result.
Equipment and supplies:

- Giemsa stain, decanted from the stock solution into a 25 ml. bottle
- Methanol
- Absorbent cotton wool
- Test tubes of 5 ml. capacity
- Distilled or de-ionized water buffered to pH 7.2
- Pasteur pipette with rubber teat
- Curved plastic staining tray, plate, or rack
- Slide drying rack
- Timing clock

Thick blood films must be completely dry before being stained. Prolonged storage before staining in humid conditions can result in fixation and poor staining.

Procedure:

1. Fix the thin film by dabbing it with a pad of cotton wool dampened with methanol, or dip it briefly into methanol. Methanol, and its fumes, must not make contact with the thick film.
2. Using a small container to hold the prepared stain, make up a 10% solution of Giemsa stain in buffered water, mixing well before placing on the slides. Each slide needs approximately 3 ml. of stain to cover it. Three drops of Giemsa stain from the Pasteur pipette added to 1 ml. of buffered water makes a 10% solution. The diluted Giemsa should be prepared immediately before staining and any excess stain discarded. Diluted Giemsa stain must be used within a maximum of 15 minutes after preparation.
3. Place slides face down on the curved tray or face upwards on the staining rack.
4. Pour the stain gently between the slide and tray until each slide is covered with stain or gently pour the stain on to the slides lying face upwards on the rack.
5. Stain films for 8 to 10 minutes. Stains vary in quality from batch to batch so the optimal staining time should be determined by staining a series of slides for varying time periods.
6. Gently flush the stain from the slide by adding drops of clean water. Do not pour the stain directly off the slides otherwise the metallic-green surface scum sticks to the film, spoiling it for microscopy.
7. When the stain has been washed away, place the slides, film side down, in the drying rack to drain and dry.

4.1.3 Screening the blood films for malaria parasites

The thick blood film should be examined to determine the presence of parasites and the parasite density. There may be difficulties in thick films in differentiating between:

- the later mature trophozoites and gametocytes of *P. vivax*,

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25 Methanol (methyl alcohol) is highly toxic and flammable; it can cause blindness and even death if swallowed in any quantity. When not in use it should be stored in a locked cupboard.
Malaria Microscopy Quality Assurance Manual - Version 1

- *P. malariae* trophozoites and rounded *P. falciparum* gametocytes, and
- the late trophozoite and gametocyte stages of *P. malariae*.

Mixed malaria infections may be difficult to identify. *Plasmodium vivax* infections are usually easy to identify because of the range of stages, red cell enlargement and the presence of Schuffner’s dots. The examiner must be constantly aware of the possible presence of another species in any positive film, usually at a lower density, and of the fact that *P. falciparum* rings remain small with no obvious effect on the host red cell. The exception to this rule is with *P. falciparum* infections in semi-immune individuals, when some rings are larger and demonstrate Maurer’s clefts in the thin film.

The initial screening should be done by two qualified microscopists, including at least one expert. These readings should be performed independently by the microscopists who should not consult one another on any of the readings. If possible parasites counts should be done on thick films and parasites should be enumerated against 500 white blood cells.

4.2 The production of Giemsa stained blood films in bulk

4.2.1 Selecting suitable donors

For ethical and other reasons, speed is important when selecting donors. Patient management and treatment should not be significantly delayed by the slide bank recruitment process and severely ill patients should not be included.

Once a patient is selected for participation, the following must take place quickly.

4.2.1.1 Obtaining informed consent when requesting donor participation

The reason for requesting a specimen should be explained clearly to the donor. He/she should sign a standard document of consent. The blood sample should then be taken as soon as possible. A time lapse of more than one hour may mean significant changes to the parasite picture and the need to re-confirm the donor’s suitability may be necessary. Thus, another blood film will have to be examined before deciding to proceed.

Alternatively, to save time and trouble to the donor, the full sample can be collected in EDTA and a decision then reached, following examination of a blood film made from that sample. This approach reduces the need for another finger-prick, and delays to the patient’s treatment, but requires consent for venepuncture for cases that may not then be used for slide preparation.

Inclusion in the programme means being able, if necessary, to:

- trace patients to their home;\(^ {26} \)

\(^ {26} \) In situations where control activities are in operation collaboration between NMCP units will require such information to be available, for possible further malaria control action.
Annex 8: National Slide Bank SOPs

- confirm selected donor’s identity and meets established criteria;
- fully inform donor, or parent/caregiver, of why a blood specimen needs to be taken;
- obtain signed consent, following national policies;
- ensure ethical (and confidentiality) issues are followed;
- bring patient and guardian to operations centre, if separate from recruitment centre;
- collect a further finger-prick specimen to confirm continued inclusion;
- make donor comfortable while confirming the diagnosis (SOP 4.1); and
- ensure donor receives treatment and is assisted to return home.

4.2.2 Collection of the specimen and preparation of replicate films and bulk staining

Equipment and supplies:

- EDTA-treated vacutainer 5 ml. capacity (Lavender top), or
- EDTA-treated tube of 5 ml. capacity, one per donor
- Disposable syringes, 5 ml.–10 ml. capacity, one per donor
- Sterile syringe needles, 2 l. or 23 gauge, one per donor
- Latex protective gloves, minimum three per worker
- Sharps container
- Sterile lancets
- Methanol
- Wrapped clean micro-slides; numbers as required
- Cover-slips (sufficient size to cover thick and thin films)
- Mounting media
- Pasteur pipettes
- Micropipettes (Eppendorf type) 2 µL–20 µL capacity
- Pipette tips
- Templates for thick blood films
- Slide boxes to store 100 slides horizontally and protect them from insects and dust
- Desiccators
- Hard plastic boxes with airtight lids to hold dried micro-slides
- Silica gel, active, for airtight boxes or desiccators
- Giemsa stain, in 25 ml.–50 ml. bottles, decanted from the stock solution
- Absorbent cotton wool
- De-ionized water buffered to pH 7.2
- Slide drying racks
- Timing clock, recording up to 60 minutes
- Binocular microscope fitted with x10 oculars, x40 and x100 objectives, a mechanical stage and an objective marker
- Mains or battery powered microscope lamp
- Immersion oil
- Tally counters, minimum 2
- Electronic calculator
- Record forms
- Pen and pencil
- Staining troughs to hold 20 slides placed back to back
Measuring cylinder, capacity 100 ml.–500 ml.
Measuring cylinder, capacity 10 ml.–25 ml.
Flask or beaker–capacity will depend on the amount of stain to be made up

Procedure

Following the precautions used when handling human blood:

1. Clean the venepuncture site with a cotton pad dampened with 70% methanol and let it air dry.
2. Using the Vacutainer, or a disposable syringe and 21–23 gauge needle, draw 3 ml.–5 ml. of blood from an antecubital vein. **Less than 3 ml. of blood gives a poor ratio of blood to EDTA and has a detrimental effect on both red cell structure and quality of staining.**
3. Transfer the blood to an EDTA-treated tube.
4. Gently invert the tube a few times to mix blood and EDTA. **Do not shake.**

Preparing blood films

**Note:**

- The following methods vary from making routine blood films so the team will need to have practised these methods in advance to ensure slide-making efficiency using the templates and fixed quantities of blood.
- Films from EDTA-treated blood should be made within one hour of being taken to preserve parasite and leukocyte morphology.77
- Keep EDTA-blood at room temperature since changes to parasite and leukocyte morphology quickly follow refrigeration.
- Schuffner's dots stain poorly the longer blood stands.
- Mature gametocytes in the sample will start to exflagelate after 15 minutes.
- Lay the pre-printed slide templates (Annex 11) on a flat, firm surface, ensuring there is room to work. The template can be modified to make a thick and thin film on one slide. Some programmes have the slides marked before purchase, but it increases costs.
- Wearing gloves, place a clean micro-slide directly on each template and handle slides only by the edges, ensuring fingers are clean and grease free.
- Thick and thin films are normally made on the same slide. Alternatively, separate slides may be prepared. In general, it is useful to prepare slides as they are normally prepared in the national programme, as this is appropriate for testing the microscopists within that programme.

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77 If ookinete stages are required, the sample is left standing at room temperature overnight. Thick and thin films are made with blood taken from just below the buffy-layer, after centrifugation.
Making thick films

One person delivers the measured amount of blood and another follows behind doing the spreading, both wearing protective gloves:

1. Using the micropipette and fitted tip, and working quickly to avoid the blood drying, deliver 6 µL of blood on to each of the indicated areas outlined in the templates. Using the corner of a clean slide, spread the blood evenly to cover all parts of the area of the template.
2. Ensure clots do not form by regular replacement of the pipette tip and spreader.

Making thin blood films

There is no need to use a template for this activity, but:

1. Working quickly, repeat the process, but this time placing 2 µL of blood in the centre of the slide on the mid-line of the slide. Spread the film using the edge of another clean slide.
2. Thin films dry quickly and are ready for placing in a box within 30 minutes of being made.
3. Although thin films can be stained as soon as they are dry, storing overnight in the desiccator, or warm cupboard, seems to enhance staining.

Drying

1. All films must dry evenly, protected from dust and insects.
2. Thoroughly dry slides horizontally in slide boxes with close-fitting doors.
3. Slides with thick films should be dried for 24 hours in a desiccator or warm cupboard as this improves blood-film adherence and staining. Poor adherence is a problem with EDTA-treated blood.

This routine is repeated for each blood sample collected. Clean utensils, glassware, and templates slides must be used for each blood sample to eliminate the risk of cross contamination.

It is important to double-check the accuracy of the recorded data, as well as the coding in the register(s) and on the slides.

4.2.3 Staining the blood films using the slow (3%) method of Giemsa staining

This method is excellent for staining slides for slide banks. The method performs best when the slides have been dried for 24 hours.
Method used for staining 20 or more thick blood films at a time

**Equipment:**

- Giemsa stain
- Methanol
- Absorbent cotton wool
- Staining troughs to hold 20 slides placed back to back
- pH 7.2 buffered water, adjusted based on the test run
- Measuring cylinder, capacity 100 ml.–500 ml.
- Measuring cylinder, capacity 10 ml.–25 ml.
- Flask or beaker, capacity will depend on the amount of stain to be made up
- Timing clock
- Slide drying rack

**Procedure:**

1. Place slides, back to back, in a staining trough with thick films at one end and thin films at the other end. The stain, and later water for rinsing, will be introduced at the thin film end. **Note:** First fix thin films by dabbing each film with cotton wool dampened in methanol, or, briefly dipping films into methanol. Do this carefully and do not let methanol or its fumes come in contact with the thick films as they quickly fix and spoil them.

2. Calculate the total amount of stain required by covering 20 blank slides in a staining trough with water and measuring the amount of water the trough takes. The total amount of stain required is calculated by multiplying the number of troughs filled with slides, by the amount of stain each trough takes (this should well be done in advance).

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

4. Gently pour the stain into each trough at the end where the thin films are. **Avoid pouring stain directly onto the thick films.**

5. Stain for 45–60 minutes. Experience will select the best time.

6. Gently pour clean water into the trough to float off the iridescent “scum”. A less satisfactory method is to gently immerse troughs into a basin of clean water; avoid picking up the iridescent scum when removing troughs from the basin.

**Note:**

With bulk staining, first test a small, representative sample to establish staining properties. This is necessary with films made with EDTA-treated blood as EDTA can adversely affect the pH. Stain about 10 thick films in a 3% fresh Giemsa solution and evaluate the staining microscopically. If necessary, adjust the pH of the buffered water, based on the microscopically visual quality of the staining. Staining that is too pink requires the buffered water to be more alkaline; too blue to be more acid. Trouble taken over this pays dividends in the end product of a mass staining exercise.
(7) Finish rinsing with clean water. The pH of water used for rinsing is important as acidic water de-stains films whereas alkaline water (pH 7.2) maintains the quality of staining.

(8) Place slides film side down in a drying rack to dry. Ensure wet thick films do not touch the edges of the rack.

(9) Dry films should be packed, face to back, and stored in a desiccator charged with activated silica gel.

**4.2.4. The transfer of parasites during staining**

Parasites may transfer between blood films during staining. It can be controlled by adding small amounts of a detergent to the stain solution.

While parasite transfer may happen, especially with larger stages such as gametocytes or schizonts, the average worker need worry little about it. The mass staining of blood films made from one donor ensures that if parasite transfer does take place it can make little difference to the result. Dirty and improperly cleaned and dried utensils have a very small risk of retaining free-floating parasites that can adhere to a film during new staining. In order to minimize these risks, never stain slides from different donors at the same time. Change all staining solutions between donors and clean the staining troughs.

**4.2.5 Care of glassware and measuring utensils**

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

After use, immediately rinse utensils used in Giemsa staining with clean water to remove the free stain. Then soak in a detergent solution before final washing, rinsing and drying. *Note: Any detergent left on glass and plastic-ware will alter the pH and affect staining quality.*

**4.2.6 Verification of blood film quality**

(1) Place the malaria blood film on the microscope stage (frosted end to the left). Switch on the light and adjust the light source optimally by looking through the ocular and the 10 times objective.

(2) Examine the thick and thin films grossly under 10 times objective to check the quality of the slide as follows:

(a) Is the thick film >90% intact? If no, do not read.

(b) Except for around the edges, the red cells should be completely lysed. If not, do not read.

(c) Are white blood cells (WBCs) on the thick films properly stained and well distributed?

(d) Do red blood cells (RBCs) in the thin film appear pink to red? If no, do not read.

(e) Does the thin film have RBCs that are in one single, distinctive layer? If no, do not read.

(f) Do the thick or thin films have significant debris? If yes, do not read.
5. Validation of blood films

5.1.1 Validation of blood films by microscopy

Each enrolled subject will have approximately 200 slides made from their whole blood specimen. Each set of 200 slides should be validated by a number of expert microscopists. The way this is done may vary. It is essential that there is very high uniformity on the production of blood films for validation of one slide to be used as sufficient for others within the set. The way validation is organized will depend on the availability of expert microscopists (the term “expert” implies that they have been prequalified and their competency confirmed), and should allow monitoring of both inter-slide and inter-reader consistency. For example:

Select 2 slides from the set of 200 for each of six expert (pre-qualified) microscopists, to evaluate for species, presence of gametocytes and parasite density (count will be reported in parasites/µL). The mean and standard deviation (SD) will be calculated from these 12 counts and all 12 counts must fall within + 2 SD of the mean. If all 12 slides meet validation criteria, then all 200 slides will be considered validated.

5.1.2 Validation of blood films by polymerase chain reaction (PCR)

Even expert microscopy is likely to miss one species in a mixed infection, as apparent suppression of one species may occur in the presence of another, resulting in a very low parasite density. However, it is essential to note the true number of species on a slide bank film, as the films are used for training and testing microscopists and it is possible a later candidate may see one of few parasites of one species present on the film, missed by the microscopy validation process.

DNA may be identified by PCR from dried blood spots on filter paper, or otherwise preserved blood samples. Different laboratories will have preferred methods. It is important to include all four of the main malaria parasites infecting humans, *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*, and where relevant to include *P. knowlesi*.

*Slide reading diagram*
(1) To determine the positive and negative film and the species present: Begin at the left upper section of the thick smear and then scan right, ensuring that the majority of the thick film is covered. A higher number of field than normal (e.g. 500) should be viewed to validate a slide bank.

(2) To determine the parasite count: The above technique is used, and parasites counted against at least 500 WBCs. All parasites in the final field are counted even if the count exceeds 500 WBCs. Final parasites counts may be calculated against a fixed number of white blood cells, as this is a common standard used in training and clinical practice, but should also be reported against the white cell count.

5.1.3 Validation by polymerase chain reaction

Principle

Recent studies have shown that nucleic acid testing (NAT) utilizing PCR achieved greater sensitivity for malaria diagnosis than with microscopy, particularly for mixed infections and low parasitaemias. Genotyping malaria parasites is proving to be a useful tool for distinguishing Plasmodium species. This SOP details the procedure for collecting DNA by applying fresh blood to DNA transport cards.

Equipment and materials:

- DNA transport card (filter paper or similar material designed for DNA preservation and transport)
- Pipette volume 20 µL–200 µL and pipette tips
- Ziplock plastic bags
- Desiccant

Specimen

Fresh whole blood, or anti-coagulated (EDTA, sodium citrate, ACD or heparin) blood

Safety precautions:

- Gloves must be worn to prevent contamination of DNA transport cards.
- Universal precautions, including relevant personal protective equipment, must be used.

Procedure

(1) Label the DNA transport card with appropriate sample ID, date of sample collection and initials of technician responsible (see diagram).

(2) Drop the blood (125 µL per 2.54 cm) onto the cards in a concentric circular motion within the printed circle area (4 circle areas for 1 patient, diagram below). Avoid
“puddling” of the liquid sample, as it will overload the chemicals on the card. Also, do not rub or smear the blood onto the card.

(3) Samples applied to DNA transport cards are ready for immediate room temperature storage. Note: If samples are to be processed shortly after application on the DNA transport card, allow the sample to dry for one hour at room temperature prior to placing in a polyethylene ziplock bag. Do not heat to shorten the drying period. The ziplock bag should contain a desiccant.

5.1.4 Permanently mounting blood films to extend shelf-life

The life expectancy of stained blood films is about two years under tropical conditions. Films regularly exposed to immersion oil, solvents and rough usage, such as when immersion oil is wiped off the film, will have a shorter lifespan. Films can deteriorate before the supervisor, or trainer realizes the slides no longer perform their purpose, and that new slides must be obtained. With correct care, slides can have a life expectancy of 10 to 20 years.

Blood films permanently mounted with a cover-slip, have an extended shelf-life. Slide banks should permanently mount important slides in their collection. There is a selection of mountants available and they can be used without fear of the oil immersion or solvents spoiling the blood film or stain.

(1) To identify particular parts of the film, a ring may be made around the specimen, using the diamond pointed objective marker. This is done on the film before the slide is mounted.

(2) Place a drop of mountant on the dry blood film. Judging the amount will require experimentation and experience. If the cover-slip is rectangular (below), an elongated “drop” of greater volume is required.

(3) A clean cover-slip, the size depending on the dimensions of the film, is placed on to the mountant and positioned over the film using a pair of forceps.
(4) Air pressure settles the cover-slip but, if more mountant is required, then another drop can be introduced from one side of the cover-slip. Very gentle pressure may be placed in the centre of the cover-slip with a finger. Ensure the minimum amount of medium is used so that drying is quick and there will be sufficient working space between the oil-immersion lens and cover-slip.

(5) Slides take about 48 hours to dry in warm conditions, but may take a number of days at cool temperatures. Ensure mountant is fully dry before packing.

(6) Slides can then be packed, in slide boxes, and used as required.
Annex 9

Model Information Sheet and Consent Form for Slide Bank Donors

Draft Minimum Standard Consent Form

- To be modified to fulfil local requirements, retaining each element below.
- Initial finger-prick blood screening may be included if this is not part of normal clinical practice for the presenting symptom.

The following have been explained to me and have been understood by me:

Purpose
The (NAME OF INSTITUTION) is developing a collection of malaria slides for the purpose of teaching and testing technicians who diagnose malaria, with the aim of improving accuracy of diagnosis. For this purpose, they request the collection of a blood sample to make a large number of identical blood films (smears), and as dried blood spots, store them to be used now and in the future for teaching and checking the accuracy of technicians’ work.

Procedures
I will have a 3 ml.–5 ml. sample of blood (1/2 teaspoon) extracted for this study. The blood will be assessed by microscopy and other tests to check the type of malaria present, and the number of parasites. If found suitable, it will be placed on slides and used to teach and check the performance of laboratory technicians in or outside (NAME OF COUNTRY). This will not affect my treatment.

Case management and treatment will be provided by the (NAME OF HOSPITAL/CLINIC) according to the result of the normal blood tests performed at the (NAME OF HOSPITAL/CLINIC).

Risks and discomfort
I understand that the risks involved in this study are minimal. They include the discomfort of drawing a sample of blood, rare bruising and infection at the site of needle stick, and very rarely, fainting. New needles will be used for each patient so there is no risk for transmitting diseases.

Confidentiality
All my identifiable records and information will be kept strictly confidential, and remain in secure storage with the (national institution and health service) collecting the blood sample. Access to personal identifying information will only be accessible to research staff of this institution directly involved this study, unless I give express written consent otherwise. Samples sent to other institutions involved in checking the type of malaria present, and all slides used for training and testing technicians, will have no personal information identifying me.

Withdrawal of consent
My participation is voluntary and I can withdraw from the study at any stage for any reason. Withdrawal or refusal will not in any way affect management of malaria or other illness.

Contact information
For further inquiries, I can contact the following persons:
1. Name, and address of collecting institution
2. Name and address of national ethics committee contact point.
Annex 9: Model Consent Form

Name: ________________________________    Name of guardian: ________________________________

Signature: _____________________________    Relationship (guardian): _________________________

(Literate) Witness: _____________________    Date: ________________________________________

Investigator’s statement: I, the undersigned, have defined and explained to the volunteer in a language he/she understands, the procedures of this study, its aims and the risks and benefits associated with her participation. I have informed the volunteer that confidentiality will be preserved, that he or she is free to withdraw from the trial at any time without affecting the care she will receive at the clinic. Following my definitions and explanations the volunteer agrees to participate in this study.

______________________________  ________________________________________________
Date                                          Name of investigator who gave the information

Signature: ________________________________
Draft Minimum Standard Consent Form

- To be modified to fulfil local requirements, retaining each element below.
- Initial finger-prick blood screening may be included if this is not part of normal clinical practice for the presenting symptom.

Collection of blood for the malaria slide bank for training and testing laboratory technicians.

Purpose
The [NAME OF INSTITUTION] is developing a collection of malaria slides for the purpose of teaching and testing technicians who diagnose malaria, with the aim of improving accuracy of diagnosis. For this purpose, they are requesting to collect a blood sample to make a large number of identical blood films (smears), and as dried blood spots, store them to be used now and in the future for teaching and checking the accuracy of technicians’ work.

Procedures
A 3 ml.–5 ml. sample of blood (1/2 teaspoon) will be extracted for this study. The blood will be assessed by microscopy and other tests to check the type of malaria present, and the number of parasites. If found suitable, it will be placed on slides and used to teach and check the performance of laboratory technicians in or outside [NAME OF COUNTRY]. This will not affect my treatment.

Case management and treatment will be provided by the [NAME OF HOSPITAL/CLINIC] according to the result of the normal blood tests performed at the [NAME OF HOSPITAL/CLINIC].

Risks and discomfort
The risks involved in this study are minimal. They include the discomfort of drawing a sample of blood, rare bruising and infection at the site of needle stick, and very rarely, fainting. New needles will be used for each patient so there is no risk for transmitting diseases.

Confidentiality
All identifiable records and information will be kept strictly confidential, and remain in secure storage with the [national institution and health service] collecting the blood sample. Access to personal identifying information will only be accessible to research staff of this institution directly involved this study, unless the donor gives express written consent otherwise. Samples sent to other institutions involved in checking the type of malaria present, and all slides used for training and testing technicians, will have no personal information identifying me.

Withdrawal of consent
Participation is voluntary and a person can withdraw from the study at any stage for any reason. Withdrawal or refusal will not in any way affect management of malaria or other illness.

Contact information
For further inquiries, please contact the following persons:
1. Name, and address of collecting institution
2. Name and address of national ethics committee contact point
Annex 10

Model Data Sheet for Slide Bank Donor

Subject ID: __________________________ Date: ____________

Instructions: Print only. Do not leave blanks. If information is not available or is unknown, indicate as appropriate, using codes below:

NMI: No middle initial  UNK: Unknown  N/A: Not applicable

1. Age of participant: __________ (Years) Year of birth: __________________________

2. Gender:  ☐ Male  ☐ Female

3. Address (Country) ____________________________________________

4. Diagnostic test done?  ☐ MBF  ☐ RDT

5. Positive diagnostic test?  ☐ Positive  ☐ Negative

6. Have you had malaria in the past 10 years?  ☐ Yes  ☐ No  ☐ Not sure

7. If yes, when? __________________________

8. Have you travelled to a malaria endemic area?  ☐ Yes  ☐ No

9. If yes, which district __________________________

10. How long was your stay?  ☐ <2 weeks  ☐ 2–4 weeks  ☐ 1–2 months

11. Have you used malaria prophylaxis in the last 2 months?  ☐ Yes  ☐ No

12. If yes, by whom?  ☐ Self  ☐ Pharmacist  ☐ Other

____________________________________________________________________

Information obtained by: __________________________ Date: ____________

(Clinic staff signature) (dd / mm / yy)
Annex 11
Template for Thick Films

Thick film template (circle) is 1.2 cm in diameter and takes 6 µL of blood. Spread evenly to edge of circle.
# Annex 12
## Example Checklist for Internal Quality Assurance

<table>
<thead>
<tr>
<th>Category</th>
<th>Checklist Questions</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Laboratory Design</strong></td>
<td>There is sufficient working surface for each member of the laboratory staff.</td>
<td></td>
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<tr>
<td></td>
<td>The electric microscope(s) is not located directly in front of a window but faces a blank wall.</td>
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<td></td>
<td>The laboratory has access to a clean water supply.</td>
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<td></td>
<td>There are hand-washing facilities.</td>
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<td></td>
<td>There is good ambient lighting at all times (including cloudy weather).</td>
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<td></td>
<td>There is an adequate electrical supply for the microscope(s).</td>
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<td>There is adequate storage space for reagents, equipment, and storage of slides.</td>
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<td>There is a safe waste management system.</td>
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<td></td>
<td>Laboratory chairs and/or stools are suitable for microscopy.</td>
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<tr>
<td><strong>Quality of the Microscope</strong></td>
<td>The microscope(s) is binocular and electrically powered.</td>
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<td></td>
<td>The microscope lamp(s) has sufficient power to provide good illumination at small aperture settings.</td>
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<tr>
<td></td>
<td>The light source can be centred.</td>
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<td></td>
<td>The microscope(s) has Plan C x100 objectives.</td>
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<td></td>
<td>Blood smears are able to be brought into sharp focus x100 oil immersion magnification.</td>
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<tr>
<td></td>
<td>The stage movement mechanism is precise and stable.</td>
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<tr>
<td><strong>Microscope Slides</strong></td>
<td>Microscope slides are clean.</td>
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<tr>
<td></td>
<td>Microscope slides are not oily to the touch.</td>
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<td></td>
<td>Microscope slides do not have scratches or surface aberrations.</td>
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<td></td>
<td>Microscope slides do not give a blue background colouration (microscopically at x100) after staining.</td>
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<td></td>
<td>Microscope slides do not have fungal contamination.</td>
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<tr>
<td></td>
<td>Slides are protected against fungal contamination (in high humidity settings).</td>
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<tr>
<td><strong>Methanol</strong></td>
<td>Methanol is Analytical Reagent (AR) grade.</td>
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<tr>
<td></td>
<td>Methanol is supplied to the laboratory in the original sealed container as supplied by the manufacturer, and is not repackaged by the supplier.</td>
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<td></td>
<td>Methanol is not oily (test – place some methanol on the fingers, it should not be sticky).</td>
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<tr>
<td>Category</td>
<td>Checklist Questions</td>
<td>Yes</td>
<td>No</td>
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<td>------------</td>
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<tr>
<td></td>
<td>There is no deformation or blistering of red blood cells in the thin blood film (this is caused by poor quality methanol).</td>
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<tr>
<td></td>
<td>The methanol used for slide fixing is stored in moisture-proof containers.</td>
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<tr>
<td>Giemsa Stain</td>
<td>Only stain prepared from high-quality Giemsa powder is used.</td>
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<td></td>
<td>Commercial Giemsa stain is supplied to the laboratory in the original sealed container.</td>
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<td></td>
<td>The laboratory has a Stain QC Register recording the batch number and expiry date of supplies received, the QC results on each batch (staining time, staining quality, optimal pH of use) and any problems encountered.</td>
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<td></td>
<td>Stock stain is stored in a tightly sealed dark glass bottle.</td>
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<td>Stock stain is not stored in direct sunlight or near a heat source.</td>
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<td></td>
<td>The stock stain used by the laboratory was prepared less than two years ago.</td>
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<td></td>
<td>Stained blood films do not contain stain precipitate.</td>
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<tr>
<td>Diluted Giemsa stain</td>
<td>Stock stain is always diluted in buffer to the correct pH.</td>
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<tr>
<td></td>
<td>The diluted stain contains no stain precipitate.</td>
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<td></td>
<td>The surface of the diluted stain does not have an oily appearance. For horizontal slide staining (using a staining rack) this is best observed after the stain has been added to the slides. This effect can be caused by poor quality methanol used to prepare Giemsa stain from powder.</td>
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<tr>
<td></td>
<td>Diluted stain is always discarded within 15 minutes of preparation.</td>
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<tr>
<td>Thick blood films</td>
<td>More than 95% of thick films have the correct thickness. It should be just possible to read newsprint through the thick film while it is still wet.</td>
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<td></td>
<td>There is flaking in the centre of the smear (a hole in the centre of the thick film) in less than 2% of the thick films.</td>
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<td></td>
<td>100% of the thick films are correctly stained.</td>
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<td></td>
<td>None of the thick films contain stain precipitate contamination.</td>
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<td></td>
<td>There a protocol for the preparation of thick films of the correct thickness from patients with severe anaemia.</td>
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<td>Slide warmers may be used with caution in high humidity settings.</td>
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<tr>
<td>Thin blood films</td>
<td>More than 95% of the thin films have a smooth semi-circular tail.</td>
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<td></td>
<td>In more than 95% of the thin films, the red cells are just touching and not overlapping in approximately 20%–30% of the film (the reading area).</td>
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<td></td>
<td>No thin films have water damage (retractile artefacts inside the red cells).</td>
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<td>Thin films are fixed immediately after drying.</td>
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<tr>
<td>Category</td>
<td>Checklist Questions</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Staining</td>
<td>The laboratory has a pH meter that reads to 2 decimal places.</td>
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<td>The pH meter is calibrated with calibration buffers according to manufacturer’s directions.</td>
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<td></td>
<td>A pH adjusted buffer is always used to prepare diluted Giemsa.</td>
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<td></td>
<td>The pH of the buffer is calibrated for each batch of Giemsa.</td>
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<td></td>
<td>Slides are always washed in water of the same pH as the buffer used for Giemsa dilution.</td>
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<td>The diluted Giemsa is always prepared in a clean measuring cylinder.</td>
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<td></td>
<td>There is an absolute rule that the diluted Giemsa stain is discarded in less than 15 minutes after preparation.</td>
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<td></td>
<td>The trophozoite chromatin stains red to “rusty red”.</td>
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<tr>
<td></td>
<td>The trophozoite cytoplasm stains blue to strong blue.</td>
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<td></td>
<td>The thick film background is stained light pink to grey.</td>
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<td></td>
<td>The red cells in the thin film are stained pink.</td>
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<td></td>
<td>The nuclear lobes of the polymorphs are stained significantly darker than the cytoplasm.</td>
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<td>Slides are always washed from the thin film end.</td>
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<td></td>
<td>All slides are washed gently by a technique that “floats” the stain off with minimal without disturbing the thick film.</td>
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<td></td>
<td>Laboratory staff who perform staining have protective clothing to protect their personal clothing.</td>
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<tr>
<td>Counting</td>
<td>The laboratory reports the actual number of trophozoites when required against 500 (200) WBC.</td>
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</tr>
<tr>
<td>Slide Reading Times</td>
<td>All laboratory staff who report malaria examination results read a minimum of 10 thick blood films each month.</td>
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<tr>
<td></td>
<td>Laboratory staff always read a minimum of 100 fields before reporting a film as negative.</td>
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<tr>
<td></td>
<td>There is no pressure on microscopists to read slides more quickly than the standard reading time (such as end of day, “urgent cases”).</td>
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<td>There is a laboratory protocol that ensures that microscopists do not continuously read malaria slides for more than 3 hours without a 30-minute break.</td>
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<tr>
<td>Species identification</td>
<td>Thin films are available for species identification where a mixed infection is suspected or species identification is unclear on the thick film.</td>
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