Technical consultation to update the WHO Malaria microscopy quality assurance manual

26–28 March 2014, Geneva, Switzerland
Meeting Report | Global Malaria Programme
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Acknowledgements

The consultation was prepared by the WHO Global Malaria Programme in consultation with WHO regional malaria advisers and representatives of agencies that provide technical support to improve the quality of malaria microscopy in malaria-endemic countries. Colleagues who contributed ideas and suggestions for the agenda and proposed technical resource persons to give presentations were Dr J. Namboze (Inter-Country Support Team, Southern and Eastern Africa), Dr E. Christophel (Regional Office for the Western Pacific), Dr L. Ortega (Regional Office for South-East Asia), Dr M.P. Ade y Torrent (Regional Office for the Americas) and Dr J. Cunningham and Dr A. Bosman (WHO Global Malaria Programme), who served as the WHO secretariat at the meeting. Helpul input was also received from Dr M. Aidoo (Centers for Disease Prevention and Control, USA), Dr L. Barat (President’s Malaria Initiative and US Agency for International Development), Mr K. Lilley (Australian Army Malaria Institute) and Dr J. Carter (African Medical and Research Foundation, Kenya).

The deliberations on external competence assessment (ECA) and its extension were based on preparatory work to define the procedures by Mr K. Lilley and Dr D. Bell (Intellectual Ventures Laboratory, USA), commissioned by the Regional Office for the Western Pacific. The discussions on the main requirements for malaria slide banks were greatly facilitated by a comparative analysis written by Ms C. Masetti (WHO consultant, Inter-Country Support Team, Southern and Eastern Africa) of the procedures used at the WHO collaborating centre in the Research Institute for Tropical Medicine (RITM) in the Philippines, which prepares the WHO reference malaria slide bank and those for six other research centres in Africa. The preparatory work was made possible by input from Dr S.O. Coulibaly (WHO Regional Office for Africa), Mr K. Lilley, Dr D. Ndiaye (Cheikh Anta Diop University, Senegal), Dr T.A. Nigusse (Columbia University International Centre for AIDS Care and Treatment, Ethiopia), Dr S. Owusu-Agyei (Kintampo Health Research Centre, Ghana), Dr W. Oyibo (College of Medicine of Lagos, Nigeria) and Ms B. Poonsamy (National Institute for Communicable Diseases, South Africa).

As part of the preparatory work for the consultation, Mr Lilley was contracted by the WHO Global Malaria Programme to undertake a survey of key developers and technical experts in the field of quality management for malaria microscopy and to collect and collate suggestions for improving the second edition of the Manual. These were presented and discussed at the meeting. In addition, Ms A. Crawshaw, intern on the WHO Global Malaria Programme, managed a web-based survey questionnaire, which was sent to 75 users, including WHO staff in regional, subregional and country offices, resident advisers of the US Centers for Disease Control and Prevention in malaria-endemic countries and representatives of many agencies participating in an African coordination network to strengthen malaria microscopy. The results of an analysis of the 22 responses to this survey were used in the discussions on use of the Manual and on expectations in the field.

The report of the meeting was prepared by Ms C. Masetti and Mr K. Lilley, who were co-rapporteurs of the consultation. The draft report was circulated to all participants, and the following provided suggestions for improvement that were instrumental in finalization of the report: Dr M. Aidoo, Dr A. Bosman, Dr J. Carter, Dr G. Gonzales (WHO Regional Office for the Western Pacific), Dr T.A. Nigusse, Dr D. Ndiaye, Ms B. Poonsamy, Dr S. Owusu-Agyei, Mr E.O. Yamo (University of Nairobi, Kenya) and Dr S. L. Wattal (Directorate of National Vector Borne Disease Control Programme, India).
WHO pays special tribute to Dr Peter B. Ogembo Obare, Kenya Medical Research Institute – United States Army Medical Research Unit, Kisumu, Kenya, who was killed in a car accident shortly after the consultation. Dr Ogembo Obare made invaluable contributions to the fight against malaria and to malaria diagnostics and will be greatly missed.

Preamble

In 2009, the WHO Regional Office for the Western Pacific on behalf of the WHO Global Malaria Programme prepared the *Malaria microscopy quality assurance manual* to support quality management systems for malaria microscopy in malaria-endemic countries. The Manual, originally recommended at an inter-regional consultation in Kuala Lumpur, Malaysia, in 2005, took several years to prepare through consensus-building among experts in the field of malaria microscopy and quality assurance. Four years after its release in all regions, the WHO Global Malaria Programme convened a technical consultation to review experience in quality management of malaria microscopy in endemic countries, including implementation of the main approaches recommended in the Manual.

This report is a summary of the discussions and consensus of the experts convened by WHO at a 3-day consultation to review and update the *Malaria microscopy quality assurance manual*. 
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AMREF</td>
<td>African Medical and Research Foundation</td>
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<tr>
<td>ECA</td>
<td>external competence assessment</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>PT</td>
<td>proficiency testing</td>
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<tr>
<td>QMS</td>
<td>quality management system</td>
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<tr>
<td>RITM</td>
<td>Research Institute for Tropical Medicine (South Africa)</td>
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<tr>
<td>SOP</td>
<td>standard operating procedure</td>
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Objectives of the WHO technical consultation

The technical consultation meeting was convened to:

- review the contents of the WHO Malaria microscopy quality assurance manual (2009) and to agree on the content of the Manual that requires improvement, as determined by technical evaluation, programme experience and implementation and evaluation of alternative approaches; and
- identify major changes required to the format of the Manual to improve its readability for the target audience, including the sequence of topics, their relative depth and length, summaries, highlights and new designs and flowcharts to make it more user friendly.

A full list of participants is provided in the Annex. All presentations are accessible online at: http://www.who.int/malaria/publications/atoz/microscopy-quality-assurance-report

Practices, challenges and initiatives to improve the quality of malaria microscopy

Current quality assurance practices for malaria microscopy and questions from the field in the WHO African, American and Western Pacific regions

Presented by Dr Josephine Namboze, Dr María de la Paz Ade y Torrent and Dr Glenda Gonzales (presentations 1–3)

Discussion

Participants considered that generic standard operating procedures (SOPs) should be available as references for national health laboratories to develop their own SOPs, defining the essential steps in malaria microscopy. SOPs could be customized by individual laboratories.

The Regional Office for the Americas had noted limitations in use of rapid diagnostic tests in the Region. Tests with greater sensitivity to detect Plasmodium vivax in patients with very low parasitaemia were required. P. falciparum with HRP2 deletion had proved to be a challenge in the Amazon area, and, as a consequence, HRP2-specific tests are not used in this region. More studies should be conducted in Africa and India, with standardized methods, to identify HRP2 deletions and to determine the scale of the problem. In countries undergoing decentralization, relocation of staff to peripheral laboratories can exacerbate the problem. The recommendations in the Manual should be considered with caution, as country situations differ widely.

WHO’s recommendation on the method for quantifying parasites should be made more explicit in the revised Manual. Some laboratories continued to use the “plus” system, whereas they should use the WHO-recommended methods for quantifying malaria parasitaemia.

In order to respond to the wide demand for training for microscopists and improving their competence in malaria diagnosis, electronic training was recommended to deliver refresher courses, which could reach a larger audience at no or minimal cost.

Criteria for selecting participants for microscopy training courses were discussed. Laboratory managers tend to be selected to participate, although performing laboratory work and training
are not their daily responsibilities. These managers therefore cannot use their skills, and their attendance at certification courses provides little or no benefit to country programmes. Inappropriate selection of participants means that the technicians who require training are not included. The challenge is augmented by continuous attrition of trained staff from the public to the private sector and limited sharing of new knowledge among laboratory personnel.

Experience on training in India was shared. Currently, pre-service training lasts 10 days and refresher training 5 days. Although training had previously been longer, 10 days’ training ensured better attendance. In most African countries, training in malaria microscopy is part of pre-service training for laboratory technicians, which lasts 2–4 years, depending on the level of qualification required. In Kenya, training for malaria quality assurance officers lasts for 10 days and refresher courses for 5 days. In the Philippines, the basic malaria microscopy course lasts 2 weeks for medical technologists and 5 weeks for non-medical technologists (e.g. midwives and village health workers).

It was generally agreed that specific recommendations for malaria microscopy quality assurance are required for areas in which elimination is the goal. In pre-elimination areas in Senegal, both rapid diagnostic tests and microscopy are now being performed, and the nitrocellulose strip of the tests is being retained for possible DNA extraction and confirmation by polymerase chain reaction (PCR). Other countries in the pre-elimination phase (e.g. South Africa) are involving the private sector in providing quality assured results.

**Country experience in quality management systems (QMS) for malaria microscopy: Honduras and Peru**

Presented by Dr Maria Luisa Matute and Dr Arróspide Velasco ([presentations 4 and 5](#)).

**Discussion**

The programme in Peru has developed an electronic platform (web-based) to exchange information on the external quality assessment programme. The Government also has a web platform for its health information system.

Cross-checking of slides in Peru is cascaded down the laboratory network, and corrective actions are supervised at the level directly above; e.g. checking at peripheral level is supervised at the national level, and that at local level is supervised at the peripheral level. Site supervision is performed according to the monitoring and evaluation plan; however, additional visits may be organized if problems are detected during cross-checking.

**Resources for malaria microscopy and laboratory quality management by the African Medical and Research Foundation (AMREF) and WHO**

Presented by Dr Jane Carter and Dr Katrina Roper ([presentations 6 and 7](#)).

**Discussion**

The links between the “stepwise laboratory improvement process towards accreditation” and the “laboratory quality stepwise implementation” initiative were clarified. The aim of both systems is to strengthen QMS in laboratories, building towards the ISO 15189 standard. The first is a project initiated by the WHO Regional Office for Africa for the African Region (originally funded by the US President’s Emergency Plan for AIDS Relief), while the laboratory quality stepwise implementation initiative is a global tool.
The stepwise initiative was established following an agreement between the Regional Office for Africa and the ministries of health of the participating countries, to assess and monitor improvement in QMS of the laboratories participating in the project. There is no clear correlation between the five-star system of the stepwise process and the four-step approach promoted by the laboratory quality stepwise implementation web tool.

It was pointed out that malaria microscopy diagnosis can be performed in basic laboratories, which may not include all the QMS components promoted by these global and regional initiatives. It was suggested that although the laboratory quality stepwise implementation tool is an excellent guide for higher-level laboratories, it might be overwhelming or inappropriate for smaller facilities. Less stringent requirements should be developed for smaller facilities, because, although quality control is required, the smaller laboratories may not meet the requirements of larger ones.

The presenters stressed the importance of promoting and building a culture of quality through these initiatives, which should be reflected in the *Malaria microscopy quality assurance manual*. AMREF has been building a photographic library of samples, including malaria blood slides, and it was suggested that it be made available online for continuous training. The WHO–US Centers for Disease Control and Prevention image bank, which is available as a CD-ROM distributed as part of the *Malaria microscopy learners guide* (WHO 2010) should also be made available online.

WHO has published a *Laboratory assessment tool* (April 2012), which provides guidance on assessing laboratories and national laboratory systems, including questionnaires. This document can also be used to guide country-specific adaptations to meet local requirements or the specifics of a disease control programme (e.g. malaria).

In a discussion on assessing the impact of several QMS interventions, it was noted that an external quality assessment programme or proficiency testing (PT) can be used to monitor proficiency over time. Some trainees were able to implement what they had been taught, but improvement was slow. In the Regional Office for the Western Pacific, country office staff follow up the recommendations after each ECA. Remote training in centres of excellence can improve the capacity of individual microscopists in optimum conditions, although, once they returned to their laboratories, they face the same challenges of poor-quality reagents, non-functional equipment, a heavy workload and lack of trust by clinicians, resulting in under-utilization of services. This may contribute to the marginal improvements in the performance of laboratories observed after training. Lack of structured supervision to identify problems and take corrective action might contribute most to slow progress in improving the quality of malaria microscopy.

### General observations on the WHO *Malaria microscopy quality assurance manual* and main changes required

**Highlights of the Manual and feedback from technical experts and users**

Presented by Dr David Bell, Mr Ken Lilley and Ms Alison Crawshaw ([presentations 8–10](#))

**Feedback from technical experts**

- The preamble to the Manual should better define its target audience, which should include health personnel at multiple levels in the laboratory diagnosis pyramid.
• As the aim of the Manual is to set recommended standards for QMS of malaria microscopy, it should state the specific standards required, and the recommended SOPs should be clearly listed. Readers should be referred to the relevant documents (available by open access). The SOPs for malaria microscopy should be published independently from the Manual and be reviewed more frequently than the Manual itself.

• The revised Manual should recommend interventions that have been shown to be feasible and effective by evidence collected in countries.

• The revised Manual should further clarify the role of external quality assessment and the issue of continued training.

• The revised Manual should give practical recommendations on quality assurance and orient programmes towards more feasible, phased implementation of QMS interventions. It should provide guidance on setting priorities for activities and guide countries in determining the minimum interventions necessary in resource-constrained settings.

• A section on PT should be added to the revised Manual, with full SOPs available in different documents. The difference between external and internal (national) quality assurance should be clearly defined, including how internal quality assurance should be implemented, the number of challenges and the types of panel. This section of the Manual, including external and internal panel testing, should be expanded to guide laboratory quality managers in quality assurance activities to be performed in each laboratory.

• It was proposed that the revised Manual follow the 12 essential elements of QMS promoted in the laboratory quality stepwise implementation tool: organization, personnel, equipment, purchasing and inventory, process control, information management, documents and records, occurrence management, assessment, process improvement, customer service, facilities and safety.

Feedback from users

A survey questionnaire was devised to determine use of the Manual and to rank the usefulness of each chapter as an indirect measure of its use. The questionnaire was web-based and was disseminated to national programmes, country advisers of the US Centers for Disease Control and Prevention, the Regional working group of the Regional Office for Africa and colleagues at the regional offices for the Western Pacific, South-East Asia and the Americas.

• Hard copies of the Manual were generally found at the offices of the national malaria control programme but not at national health laboratories.

• In all places where microscopy services are provided, a functional QMS should be in place.

• The revised version of the Manual should target both the public and the private sector. In some countries, the private sector is an important source of microscopy for malaria but has little interaction with public health services. Collaboration with the private sector should be strengthened, and QMS interventions and recommendations should apply to both settings.

• The Manual should cover the “what” rather than the “how” of activities. As far as possible, the private sector should implement the same practices as the public sector. The manual on Universal access to malaria diagnostic testing (WHO 2011) covers the role of rapid diagnostic tests and microscopy in countries and will not be covered in this Manual.
- The levels of microscopy competence described in the Manual for ECA target a core group of highly competent malaria microscopists (i.e. the national core group). In order to extend competence assessment in a country, lower standards should be included in the Manual as according to the clinical relevance of their diagnostic skills.

- In many peripheral health centres with a laboratory, there are no inpatient facilities for management of severe malaria cases. They conduct only diagnosis, pre-referral treatment and referral to higher facilities.

- Where competence in determining parasite density is important (e.g. in monitoring for drug resistance), the ranges used to assess deviations from the “real count” on reference slides should be maintained as recommended (i.e. ± 25% of parasite density). A manuscript by M. Gatton provides the statistical justification for using ± 25% as the appropriate range.

- The Manual should cover other determinants of poor diagnostic performance in the field, particularly factors not related to the competence of microscopists but to the supplies of water and electricity, equipment (microscopes, pH meters), quality of reagents, workload and influence of clinicians. These aspects should be addressed from national to peripheral level.

- A table should be provided, listing the benefits and limitations of each technique to further guide decisions on whether to adopt and implement them.

- Case studies or scenarios could be introduced into the new Manual, illustrating experience and guidance in different transmission settings.

External competence assessment (ECA) in malaria microscopy

Experience in implementation of ECA in malaria microscopy in the WHO Western Pacific, South East-Asia and African regions and SOP of the Regional Office for the Western Pacific for its expansion

Presented by Mr Ken Lilley, Mr Yamo Ouma and Mr Ken Lilley (presentations 11–13)

Discussion

National core group microscopists who undergo ECA for malaria microscopy play an important role in training (and assessing) microscopists at lower levels and should ideally be certified at level 1 or 2. Therefore, it was considered important to offer regular regional assessment of the competence of the core group.

In some ECA courses held in the WHO African Region, a significant proportion of participants were from research institutions rather than from national reference laboratories or public health programmes. While the participation of microscopists from research institutions is acceptable, they should represent only a minority of participants unless they are likely to be involved in national public health activities such as national competence assessment and training or therapeutic efficacy monitoring. It was suggested that the revised Manual should clearly state the criteria for participating in ECA to ensure that the most relevant people are assessed.

The Regional Office for Africa uses the same criteria to assign competence levels as the Regional Office for the Western Pacific; however, the Western Pacific and South-East Asia
regions have more level-1 microscopists, as the malaria quality assurance systems in the countries of the Western Pacific Region are more mature. The ECAs of the regional offices for both the Western Pacific and Africa assess competence in parasite species detection. The Regional Office for the Western Pacific has seen improved competence in parasite quantification but decreased competence in species identification. Other reasons for the difference in the competence levels in the Western Pacific and African regions could be the loose entry criteria for participants in ECA workshops in the latter and the lack of career support for microscopists with developed competence.

In some countries (e.g. Mozambique), dramatic improvements were seen in the competence of microscopists, as they had undergone training just before the ECA course. In other countries (e.g. Botswana), this was not the explanation. There was consensus that emphasis should be placed on strengthening continuous in-country refresher training instead of pre-ECA refresher training, as any improvements seen could be artificial and due only to time-limited external influences.

Follow-up activities to ECA were described. It is the role of ministries of health to verify their implementation, although this was recognized as a weak part of the programme.

The 3 years required for accreditation is long, and competence might be either lost or improved within this time. If resources allow and if the level is low (levels 3–4), microscopists should have the opportunity to participate again in ECA within 12 months.

Expansion of ECA should allow some independence between regions (e.g. the African from the Western Pacific); however, the WHO Global Malaria Programme should monitor the outcomes to ensure comparably high quality. There is a growing need to certify more microscopists in ECA courses.

Currently, 10 min are allowed for examination per slide during ECA, and participants rarely report not having enough time. The Regional Office for the Americas increased the minimum number of high-power fields that must be read on a thick film before declaring it negative to 500, because of low parasitaemia. Although more time might be required clinically, 10 min proved to be effective for the assessment.

The roles of the Asian Collaborative Training Network for Malaria in the Western Pacific and South-East Asia regions and the operational costs involved were discussed. Its roles include liaising with the two regional offices, national malaria control programmes and ECA facilitators to set priorities and plan the calendar of ECA activities for the year and to maintain the database of microscopists and their ECA results. The cost of coordinating ECA is minimal, as this is done mostly virtually. The WHO intercountry support team for eastern and southern Africa currently assists AMREF, playing a similar role to that of the Asian Collaborative Training Network for Malaria for organizational purposes. Extension of ECA in the WHO Region for Africa will require that an organization take on the roles of this Network. The Regional Office for Africa would be unable to play the key organizational role, as it is currently overstretched, with limited human resources.

In order to avoid misunderstanding about competence levels 1–4, it was recommended that WHO ECA provide accreditation only to levels 1 and 2 and that a different certificate of participation be given to participants with levels 3 and 4, stating the level of certification.

It was noted that not all competent microscopists are good trainers, as additional skills are needed to share knowledge effectively. It was suggested that ECA in malaria microscopy be complemented by training sessions on communication, teaching skills and knowledge
management, so that level-1 microscopists could become effective facilitators in national ECA schemes.

The ECA course has been limited to 12 participants for logistical reasons but could potentially accommodate larger groups.

Species identification and parasite quantification remain the areas in which strengthening is needed. Reservations were expressed about inclusion of *P. knowlesi* in the packages and standard ECA slide sets for training in regions other than the Western Pacific.

Concern was expressed about the relevance of investing resources in ECA when the majority of participants achieved a level of 3 or 4. It was suggested that national accreditation systems (national competence assessment) be established to assist in the selection of participants for ECA. For example, in the Philippines, only those who have reached level A in the national competence assessment are sent to participate in ECA. Access to a “soft” form of training might help to raise the level of competence before participation in ECA (as is done e.g. in Liberia).

All comments and suggested changes to the draft SOP for ECA in malaria microscopy should be sent to Dr Glenda Gonzales.

**National competence assessment**

**Discussion**

The revised Manual should include guidance on national systems for accreditation of competence in malaria microscopy, possibly building on successful experience. In the Philippines, ECA was conducted for the national core group, which then trained in the national accreditation system. The levels of accreditation in the national competence assessment are A, B, C and D, to differentiate them from levels 1–4 in the international ECA.

The revised Manual should define the expected roles of microscopists at each level of the diagnosis pyramid.

National competence assessment programmes could use less stringent criteria for reading slides, perhaps including possible artefacts and parasites modified by the effect of antimalarial drugs.

**Malaria microscopy reference slide banks**

**Experience of RITM Philippines in using WHO SOPs for malaria slide banks**

Presented by Dr Glenda Gonzales (presentation 14)

**Experiences and specificities of malaria slide banks developed by the Ethiopian Public Health Institute, the University of Lagos, the South African National Institute of Communicable Diseases, Kintampo Health Research Centre, AMREF and Université Cheikh Anta Diop**

Presented by Dr Tesfay Abreha, Dr Wellington Oyibo, Ms Bhavani Poonsamy, Dr Seth Owusu-Agyei, Dr Jane Carter and Dr Daouda Ndiaye (presentations 15–20)
Discussion

Some malaria slide banks were developed specifically for ECA or national competence assessment of malaria microscopists. Minimum required standards should be established for supranational and national malaria slide banks. The SOPs will depend on the competence level targeted.

The validation process, considered to be the most resource-intensive phase, might be adapted to use of malaria slide banks. For instance, in situations in which parasite quantification is not considered to be critical (e.g. in training of microscopists at lower levels), the number of validators could be reduced.

With regard to the regional slide bank in the Regional Office for the Western Pacific, as the requirements in terms of species and counts for assessments and training are different, RITM decides which cases to use for ECA and for training. ECA requires more difficult, low-density cases, while training courses have more general requirements, including a full range of counts of different species. For the regional external quality assessment, RITM assembles a mix of cases from the latest collection, which have not been used or lent; however, the validation process is the same, which is 24 readings (two copies of each sample) from 12 level-1 microscopists (six national and six international).

The experience of Ethiopia demonstrated that participants in training sessions may question the species identification and parasite quantification on the slides and challenge the trainer. The slides currently in the malaria slide bank have not been validated by PCR, whereas PCR validation would help trainers to remove any doubt about the samples used and increase their credibility. In Ethiopia, national training focuses on *P. falciparum* and *P. vivax*, which are both clinically important in the country. The slides being used in current training (training trainers and basic malaria microscopy) are not part of the slide bank, but attempts have been made to standardize the training slides to comprise *P. falciparum*, *P. vivax*, negative and *Borrelia* spp., and all the species are characterized and quantified by level-1 and level-2 experts in the country. Once the slide bank is finalized, the validated slides will be used for ECA in malaria microscopy, national competence assessment of malaria microscopists, training of trainers, basic training and external quality assessment by PT.

It was argued that the standards of the slides should be the same at national and international level; however, consensus was reached on different standards, as the slide bank for ECA in malaria microscopy malaria should reduce variation among batch slides to the minimum, while national slide banks could include slides with parasites modified by antimalarial medicines and artefacts (like routine samples).

If a malaria slide bank similar to that of the RITM was to be built in Africa, its primary use should be for ECA in malaria microscopy, followed by training and perhaps PT. Interventions and cost should be taken into consideration in making feasible recommendations for the development of a malaria slide bank. Too stringent SOPs could hinder efforts to extend the availability of a malaria slide bank to support capacity-building at country level.

As mixed infections are common in some regions, all positive samples should be confirmed by PCR. Use of PCR for negative samples might depend on the patients recruited (e.g. in a non-endemic country); however, the participants reached consensus that all negative samples should be validated. A debate on handling discrepancies in the results of PCR and microscopy was initiated. It was pointed out that validated PCR protocols were necessary (the US Centers for Disease Control and Prevention have validated their PCR protocol). Dilution of highly positive samples would automatically reduce the number of samples to be validated by PCR,
reducing the cost of PCR validation. RITM uses WHO collaborating centres (e.g. the Institut Pasteur Cambodia, the Australian Army Malaria Institute) for PCR validation at no cost to the RITM. It was suggested that regional networks or WHO collaborating centres could be engaged to conduct PCR validation for slide banks.

**Critical and non-critical steps in SOP procedures for developing malaria slide banks**

**Presented by Chloé Masetti** *(presentation 21)*

A desk review of the SOPs for malaria slide banks of the following research institutes was conducted: RITM (Philippines), the Ethiopian Public Health Institute, University College of Lagos (Nigeria), the National Institute for Communicable Diseases (South Africa), Kintampo Health Research Centre (Ghana), AMREF (Kenya) and Université Cheikh Anta Diop (Senegal).

As the RITM malaria slide bank is the only one certified by WHO and is used officially to produce the slide panels for the WHO ECA in malaria microscopy, their SOPs were used as the “gold standard” for the comparison. Each step of the procedure has been classified as “critical” or “non-critical”, depending on whether it affects the final quality of the slides to be included in the slide bank. The critical steps were discussed in plenary in order to build consensus.

A request was made that a video on malaria slide bank preparation be made available online.

**Discussion**

**Donor screening and selection**

It was agreed that patients who had taken antimalarial agents in the previous 2 weeks should be excluded from the programme in order to reduce variation, as these drugs affect the morphology of the parasites. Artefacts should be avoided as much as possible, as they are difficult to standardize across batches. Slides from patients who have taken antimalarial agents or with artefacts may nevertheless be included but only for training purposes (not for competence assessment), as microscopists are likely to encounter such malformed parasites in their places of work and should be able to identify them.

**Venous blood collection and batch preparation**

The ratio of anticoagulant to volume of blood should be standardized. The recommended maximum time between blood collection and batch preparation should be 4 h. Quality control by analysing the morphology of parasites can guide a decision to store samples longer.

It was agreed that a cooling box should be used for transport and for storage of blood samples in the laboratory before batch preparation (4–8 °C). The temperature during slide preparation should be 20–30 °C.

The RITM method for reducing variation among slides by mixing the whole blood sample six times every 20 slides should be adopted, as should the RITM method of introducing four quality control slides every 100 slides.
Batch staining of malaria blood films with Giemsa stain

Fixation of a thin film should take $\leq 3$ s, with precautions to avoid thick film fixation. The fixed slides should be dried in a flat position for 2 min. The staining time depends on the quality of the water and should be assessed in each country. It will be approximately 45 min in 3% Giemsa stain diluted in buffer at pH 7.2.

Examination of Giemsa-stained films

The recommended minimum number of high-power fields to be read to assess whether a thick film is negative is 200. All samples, both positive and negative, should be confirmed by PCR. It was agreed that the WHO-recommended method for calculating parasite density should be used.

Mounting and labelling films

The RITM method for quantifying the amount of medium to be used per slide should be adopted. Before the batch slides are mounted, quality control of the medium should be performed to ensure the right transparency and viscosity. It was agreed to adopt the RITM labelling system, which includes the year of collection, country, area and donor code. It was considered essential to introduce bar coding systems for categorizing slides.

Validation process

The minimum requirements for validating a batch of slides were established as six level-1 microscopists reading two slides per sample for a total of 12 readings. All samples should be validated by microscopy and by PCR for positivity, negativity and species identification. If there is discordance between level-1 microscopist validators and/or between microscopy and PCR with regard to positivity and species identification, the slide cannot be used for ECA.

Results of DNA amplification of venepuncture samples in comparison with dried blood spots

Presented by Mr Micheal Aidoo (presentation 22)

Discussion

The concentration and quality of DNA extracted from dried blood spots and from whole blood was discussed. The limit of detection of PCR is also related to the amount of template in the sample (target availability), with an estimated threshold of detection of 1 parasite/$\mu$L. False-positive results with PCR are considered extremely rare, unless they are due to contamination.

The advantages of using whole blood versus dried blood spots are being studied at the US Centers for Disease Control and Prevention. The results are not yet available.

It was considered that not enough information is available on the optimum storage conditions of plasma to set a standard, although freezing down a pellet could be a solution. Recommendations should be made about the type of paper or FTA cards to be used for collecting dried blood spots as well as the volume required. Dried blood spot samples in a sealed plastic bag with a desiccant can be confidently stored for about 2 months at room temperature.
Proficiency testing for malaria microscopy

Proficiency testing (PT) for malaria microscopy: experience from the National Institute of Communicable Diseases (South Africa) and AMREF

Presented by Ms Bhavani Poonsamy and Dr Jane Carter (presentations 23 and 24)

Discussion

The current Manual does not have a section on PT, although the demand is increasing. Both PT and cross-checking are important components of quality assurance, as they measure different things.

Guidelines should be available on PT in countries, with standardized slides. Ethiopia tried to establish national PT but had to abandon the initiative because of the lack of a validated malaria slide bank.

It was suggested that PT could be used to monitor the competence of WHO-accredited microscopists, as follow-up. The focus of PT is currently on the competence of laboratories and not individuals. Distance learning systems should be offered for continuous capacity-building.

In low-transmission areas, PT is required to maintain competence and awareness about malaria, but questions were raised about its relevance and modalities in settings with moderate-to-high transmission.

The panels used in PT should be analysed in the same way as routine samples in order to assess the competence of a laboratory to provide a correct diagnosis.

As PT schemes require regular implementation and heavy investment, it was agreed that they should be implemented at country level only when a mature QMS is in place.

One innovation is that of AMREF, in which the East African Regional External Quality Assessment Scheme has been using a bulk SMS system to inform laboratories about the arrival of PT panels and about deadlines. Furthermore, AMREF provided an opportunity for laboratories participating in their PT scheme to submit results online. A minority of laboratories has used this option (< 10%), but the number is slowly increasing. Although improvement in performance is slow, laboratories that participated more than four times in the Assessment Scheme had significantly better results than those participating less often. Furthermore, the increase in the number of laboratories participating in the Scheme automatically dilutes the cumulative success.

The incentives for participating in PT schemes were discussed. Laboratories involved in the “stepwise laboratory improvement process towards accreditation” could improve their level by subscribing to external quality assessments; for those undergoing accreditation, this is a mandatory requirement. The laboratory also receives certificates of participation, and the results of PT can be used by laboratory managers to mobilize resources, such as better equipment.

As a culture of quality is built across laboratory networks, the importance of participating in external quality assessment programmes should be emphasized.

The importance and sensitivity of the results of PT schemes were discussed. The results of AMREF are sent to laboratory supervisors to advocate for improvement, and educational material is sent to all the participating laboratories. It is the ministry of health, however, that
must take action to address the challenges identified. Supportive supervision might be an entry point for corrective actions after PT results and for delivering the panels.

The East African Regional External Quality Assessment Scheme started as a free-of-charge service for peripheral facilities in the East African region, but introduction of a participation fee is being discussed. The private sector and nongovernmental organizations currently pay US$ 200 per year.

AMREF was asked whether it would be possible to participate in only one discipline, but this was considered to be difficult in terms of feasibility.

With regard to the AMREF PT scoring system, the ministries of health involved in development of the system asked that the marks of participants be downgraded if the mistakes they had made could have serious clinical consequences.

The non-response rate to the National Institute of Communicable Diseases–WHO PT scheme remains high, at 20%. The Institute would consider a 10% rate to be more acceptable. The National Institute of Communicable Diseases–GlaxoSmithKline PT programme was set up to support research and was modified to assess individual microscopists instead of laboratories.

The Regional Office for the Western Pacific shared its experience in the Philippines, where panels from the regional slide bank (at RITM) are sent to national laboratories. A single or unified report, regardless of the number of microscopists who analysed the slides, was required from laboratories. Laboratories could report either the consensus results for their microscopists or the results of the most senior or experienced microscopists. Junior microscopists could read slides outside the PT programme as a training tool.

Cross-checking of malaria slides

**Slide validation or cross-checking: the experiences of India and Ethiopia**

Presented by Dr Suman Lata Wattal and Dr Tesfay Abreha ([presentations 25 and 26](#))

**Discussion**

The currently recommended slide cross-checking method does not take into consideration the elements of slide preparation and staining, which influence the quality of slides. These should be monitored, as e.g. re-use of slides influences their final quality. The method for slide cross-checking in the current Manual requires sampling of five low parasite-density positive slides and five negative slides. It was emphasized that this method is not appropriate for low-transmission settings (i.e. less than 20% slide positivity rate), and a different sampling method is required for such areas. Furthermore, facilities in which parasite density is not estimated (low-level health facilities with no inpatient capacity) or where the “plus” system is still in place require guidance on selecting positive slides for cross-checking.

Cross-checker competence is a key element in the success of a cross-checking system. The group agreed that it was preferable to read fewer slides but spend more time on each and for supervisors to provide immediate feedback for corrective action to be taken.

False-negative slides are becoming a problem in the Region of the Americas, where endemicity is decreasing.
In India, cross-checking is recognized as an important component of monitoring the quality of malaria microscopy. Data from the cross-checking system in India shows different trends in different states. Supervisory visits by national, regional, state and district officers to laboratories made it possible to identify problems, through on-site checking of prepared slides, the availability of SOPs, the quality of stains, the functioning of microscopes and interaction with microscopists and technicians, and to propose corrective measures to improve the quality of microscopy services.

With regard to the sample size for validation, India had promoted a system to cross-check 100% of positive and 10% of negative slides. Owing to the large number of slides produced each year (> 100 million), however, the system became highly demanding in terms of resources. It was subsequently revised to 100% of positive and 5% of negative slides in highly endemic areas and 100% of positive and 2% of negative slides in areas of low endemicity. Frequently, incorrect readings were associated with poor staining, reuse of slides and improper smear preparation because of lack of training. India is now looking forward to upgrading its malaria microscopy quality assurance system, on the basis of the major components of the WHO guidelines.

In Ethiopia, cross-checking is done every 4 months. This was found to be important for monitoring and upgrading the skill of peripheral microscopists but had proved to be resource-intensive (both financially and in terms of human resources) and is not readily upgraded, as the country is vast and has over 3000 health facilities. Blind cross-checking is considered valuable and could be conducted in the future with onsite supervision twice a year to extend it to as many health facilities as possible.

Cross-checking is being introduced gradually in Kenya and is considered a successful intervention.

Cross-checking in general is an important intervention for monitoring quality over time.

**Outreach training and supportive supervision**

**Outreach training and supportive supervision to improve performance of malaria microscopists**

Presented by Dr Nicole Whitehurst (presentation 27)

**Discussion**

The reasons for changes over time in the quality of slide preparation and in performance in slide reading were discussed. In countries with weaker quality assurance systems, any improvement appears more impressive than in countries that already has fairly good performance.

Outreach training and supportive supervision is a valuable intervention, but only a small proportion of laboratories are involved in the current effort. For countrywide application, the cost implications and feasibility must be considered.

One of the advantages of outreach training and supportive supervision is the teaching element, which targets both microscopists and clinicians. This intervention can address issues not only in laboratories but also in health facilities as a whole. At the end of each visit, clinicians and technicians sit together and discuss ways to improve their work.
Some areas still require clear guidance on implementing outreach training and supportive supervision, including the competence of supervisors and the number of visits. It was reported that not all supervisors have the cross-cutting knowledge and competence necessary to provide onsite support.

Integrated supervision might be a more effective way to use human and financial resources. Although it remains a good idea in theory, implementation faces a number of challenges. One of the main challenges is that limited time would be allocated to supervise malaria diagnoses, compromising the beneficial effects of the exercise. Examples of possible benefits include sharing vehicles and combining clinical and laboratory supervision. Integrated supervision could also provide information on the functionality of health facilities, and the recommendations could help in targeting vertical supervision. Integrated supervision could include direct observation of all techniques in a rotation system, with each supervisory visit having a different focus.

Monitoring implementation of a quality management system for malaria microscopy

**Rapid assessment survey of QMS for malaria microscopy in Benin and Ghana**

Presented by Mr Timothy P. Finn (presentation 28)

Discussion

Rapid assessment surveys could be additional tools for periodic assessment but require further validation. Both Benin and Ghana have a fully scaled up supervision programme, and the Ghana facilities are targeted by outreach training and supportive supervision. A range of facilities was selected for this exercise, independently of their enrolment in outreach training and supportive supervision. The tool could be used to derive a snapshot of the situation on the ground.

**SOPs for malaria microscopy collated by Regional Office for the Western Pacific**

Presented by Dr Glenda Gonzales (presentation 29)

Discussion

The group considered that generic SOPs are highly relevant and their availability is an essential step in improving quality. Generic SOPs should be validated in countries and ultimately by the WHO Global Malaria Programme. They will be available online and in hard copy in multiple languages.

SOPs are needed for the procedures other than the list proposed, including quality assurance.

In a discussion on whether SOPs should “evolve” or change over time, one of the arguments in favour of change was the incorporation of new understanding about what influences the quality of diagnosis by malaria microscopy, such as filtering Giemsa stain.

As manuals take longer to be reviewed, SOPs should be published separately.

Although SOPs may be adapted to each laboratory (e.g. equipment available), the essentials should remain the same.
A sub-group of this consultative group will be requested to review the SOPs, which will be linked to the new version of the Manual. They should be reviewed periodically, every 1–2 years.

Specific changes suggested for the new edition of the Manual

Audience of the Manual

From national malaria control programmes and national health laboratories to lower levels.

Minimum requirements

Define the role of each level:

<table>
<thead>
<tr>
<th>Level</th>
<th>High-quality equipment, supplies, supply chain, maintenance</th>
<th>Competence assessment</th>
<th>Training</th>
<th>Supervision</th>
<th>SOPs, bench aids, Manual</th>
<th>Internal quality assurance (materials including positive and negative controls)</th>
<th>Record-keeping and reporting forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supranational</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>National</td>
<td>X</td>
<td>ECA, PT</td>
<td>Training of trainers</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Regional</td>
<td>X</td>
<td>National competence assessment, PT</td>
<td>Trainees</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Facility</td>
<td>X</td>
<td>X</td>
<td>Training</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Other considerations

- electricity and facility limitations;
- delivery of ECA by an independent body;
- clarification of roles and responsibilities throughout the Manual;
- structuring by level: supranational, national, regional or provincial, facility, community;
- guidance on the minimum required package of malaria QMS activities;
- linkage of the new Manual to the 12 essentials of QMS and reference to the 12 elements of the ISO 15189 laboratory accreditation system;
- consideration of resource requirements and a phased approach for each intervention;
- placing priority on building a national core group of microscopists; and
- PT for the national level and potentially for the regional level.
Structure

- introduction, with link to ISO
- roles, responsibilities, levels
- scope
- general principles and framework
- elements (explain interventions) by level and by type
- plan of action (phased implementation)
- epidemiological issues

Elements

- equipment and supplies (high-quality reagents and equipment)
- training
- competence assessment: ECA, national competence assessment, supervision
- external quality assessment: cross-checking, PT, supervision
- internal quality assurance
- recording and reporting

Include

- chapter on PT and national competence assessment;
- cross-checking of slides, with the six parameters for quality (e.g. in Peru);
- supervisory checklist by level;
- a section on impact evaluation for each intervention, based on continuous monitoring;
- expanded chapter 10 on internal quality assurance, for accreditation and building a culture of quality;
- establishment of a national steering group, including the national core group, directorate of laboratories, the national malaria control programme and other relevant organizations (and their respective roles);
- chapter on implementation of quality assurance.

Annexes for the new version of the Manual

- Remove annex on SOPs and cross-reference them.
- Maintain annexes 1, 4, 5 (including the main body), 6, 7 and 12.
- Remove annexes 2, 3 and 11.
- Perhaps add an annex on national competence assessment and PT (e.g. types of panels, scoring system).
Implementation plan, priority (phased approach)

The colours indicate the order of introduction of activities: blue, core; green, second step; orange, third step, mature QMS.

1. Baseline situation analysis (Table 3, p. 21): resources available in the country and gaps in commodities and infrastructure
2. Identify the national core group of microscopists (undergoing ECA and qualifying as level 1 or 2).
3. Establish a national steering committee.
4. Policies, guidelines, SOPs: commodities and infrastructure
5. Competence assessment
6. Training
7. Supervision (outreach training and supportive supervision)
8. Cross-checking
9. PT
10. On-site evaluation

Monitoring and evaluation (recording and reporting) should be implemented in all phases.

Review of comments on the Manual sent before the consultation

- No comment on boxes 1–5, 7 and 8.
- **Box 6**: The infrastructure of a QMS has no relation to universal coverage.
- **Box 10**: The group reached consensus on the term “microscopist”, which should be clearly explained in the glossary of the Manual.
- **Cross-checking**: The number of slides to be cross-checked should be reconsidered. Scenarios: In a laboratory that does not quantify parasites, how do you select the five low parasite-density slides? Statistical significance for elimination settings.
- **ECA range of parasitaemia**: ECA revision of the ranges of parasite counts: minimum, 100 parasites/μL and maximum 100 000 parasites/μL.
- **Assessing deviation from true count**: It was agreed that a fixed range should be used to assess parasite density.
- **Parasite quantification**: Encourage phasing out of the “plus” system.
- **ECA certification**: Levels 1 and 2 will obtain certification, levels 3 and 4 will receive only a certificate of participation (mentioning the levels).
Final conclusions and recommendations

General recommendations for the Malaria microscopy quality assurance manual, version 2

- The Manual should set the quality standards for malaria microscopy quality assurance and quality control, which should apply to both the public and the private sector.
- The preamble to the Manual should clarify the target audience and where it is in the health pyramid.
- The Manual should provide practical guidance on phased implementation of activities (step-wise approach).
- The minimum package of activities to be implemented should be clearly defined.
- For each activity, methods for assessing their impact should be clearly described.
- The pros and cons of each activity should be clearly stated.
- As activities may vary in different epidemiological zones, case studies should be included to guide countries.
- Generic SOPs will be developed by the Regional Office for the Western Pacific and made available online (in several languages). A subgroup of the participants at this consultation will be asked to review them before they are published by the WHO Global Malaria Programme. The Manual should not include SOPs, which will be published separately.

Training and certification

- E-learning platforms should be developed to provide continuous training to a larger pool of health workers, with a photographic library online from AMREF and the WHO–US Centers for Disease Control and Prevention and the slide bank video.
- The criteria for choosing participants for training and ECA should be clearly stated.
- The certificates for ECA will be: a competence certificate for levels 1 and 2 microscopists and a certificate of participation for levels 3 and 4 (stating their score).
- WHO will establish a list of focal points that have the ECA results for each region.
- The impact of training over time should be assessed and monitored.
- The pros and cons of training under optimum conditions and of training in the field under actual conditions in facilities should be clearly described.
- The minimum competence requirements at each level of the diagnosis pyramid should be clarified in view of their clinical importance.
- National certification systems should be strengthened on the basis of the experience of the Philippines system, with grades from level A to level D.

Supervision, outreach training, supportive supervision and cross-checking

- Systems should be established for continuous assessment of supervisors and/or the competence of cross-checkers.
- The results of PT and cross-checking should guide supervision and indicate the corrective action to be taken.
• Cross-checking is highly demanding in terms of human and financial resources, and new approaches (e.g. validation during supervision) should be considered.

• The sample size for cross-checking stated in the Manual cannot be applied to countries in the malaria elimination stage and should be revised.

• The six parameters for assessing the quality of slides should be stated in the Manual, based on the experience of the programme in Peru and the US President’s Malaria Initiative for outreach training and supportive supervision.

• The Manual should state that one advantage of outreach training and supportive supervision is the involvement of clinicians in strengthening diagnosis.

• The Manual should note that integrated supervision remains difficult to implement.

Proficiency testing (PT)

• The Manual should contain a section on PT.

• The incentives for a laboratory to participate in a PT scheme include “stepwise laboratory improvement process towards accreditation”, levels and certificates.

• Countries that are considering introduction of PT must first establish a strong national malaria slide bank in order to avoid its failure (e.g. in Ethiopia).

• PT and cross-checking are both important activities, as they measure different aspects of quality assurance.

• PT is an important activity in countries in the elimination phase, where microscopists rarely see positive cases.

• Access to and use of the results of PT should be considered carefully for the success of this intervention.

• The revised Manual will include the experience of AMREF and the National Institute of Communicable Diseases on grading of PT results (including clinical relevance).

• Use of PT panels during supervision should be considered for direct correction and training.

• The PT panels could include slides with drug-affected parasites.

Malaria slide bank

• The purpose of a bank should be defined in order to determine the stringency of the SOPs.

• Slides in a national malaria slide bank could contain artefacts and parasites affected by antimalarial agents, which should be used for training purposes.

• Variation among slides in the same batch used for ECA should be reduced to a minimum.

• The validation process should include a minimum of six level-1 validators per sample.

• All samples should be validated by PCR.

• Samples in which there is a discrepancy between microscopy and PCR results can be used for training purposes but not for ECA.

• Use of regional centres of excellence for PCR validation should be explored.

• Consensus was reached on the minimum requirements for a malaria slide bank.
• Recommendations should be made about the type of FTA cards to be used for dried blood spots and about the volume of blood to be collected.

• The possibility of sharing slides among institutions, countries and WHO regions should be further discussed and established by formal agreements (memoranda of understanding).

• Samples may be diluted, if their quality is not affected, for the creation of a malaria slide bank for both training and ECA; for example, the RITM is currently not diluting slides because of agglutination issues.

Internal quality control and QMS

• This section of the Manual should be expanded.

• Reference should be made to the elements of ISO 15189.

• The revised Manual should refer to more detailed documents to guide comprehensive QMS.

Other issues discussed

• Innovative approaches should be considered and documented, such as the introduction of web-based feedback systems (e.g. in Peru, the National Institute of Communicable Diseases and AMREF), and reminders of deadline could be sent through a bulk messaging system (PT AMREF).

• Rapid assessment surveys could be an additional tool for periodic situation analyses but require further evaluation.

• Consensus was reached that the WHO Global Malaria Programme should oversee extension of ECA and play a key role in monitoring the maintenance of high-quality standards.

• In the WHO African Region, an organization must be identified to manage ECA for malaria microscopy.
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