

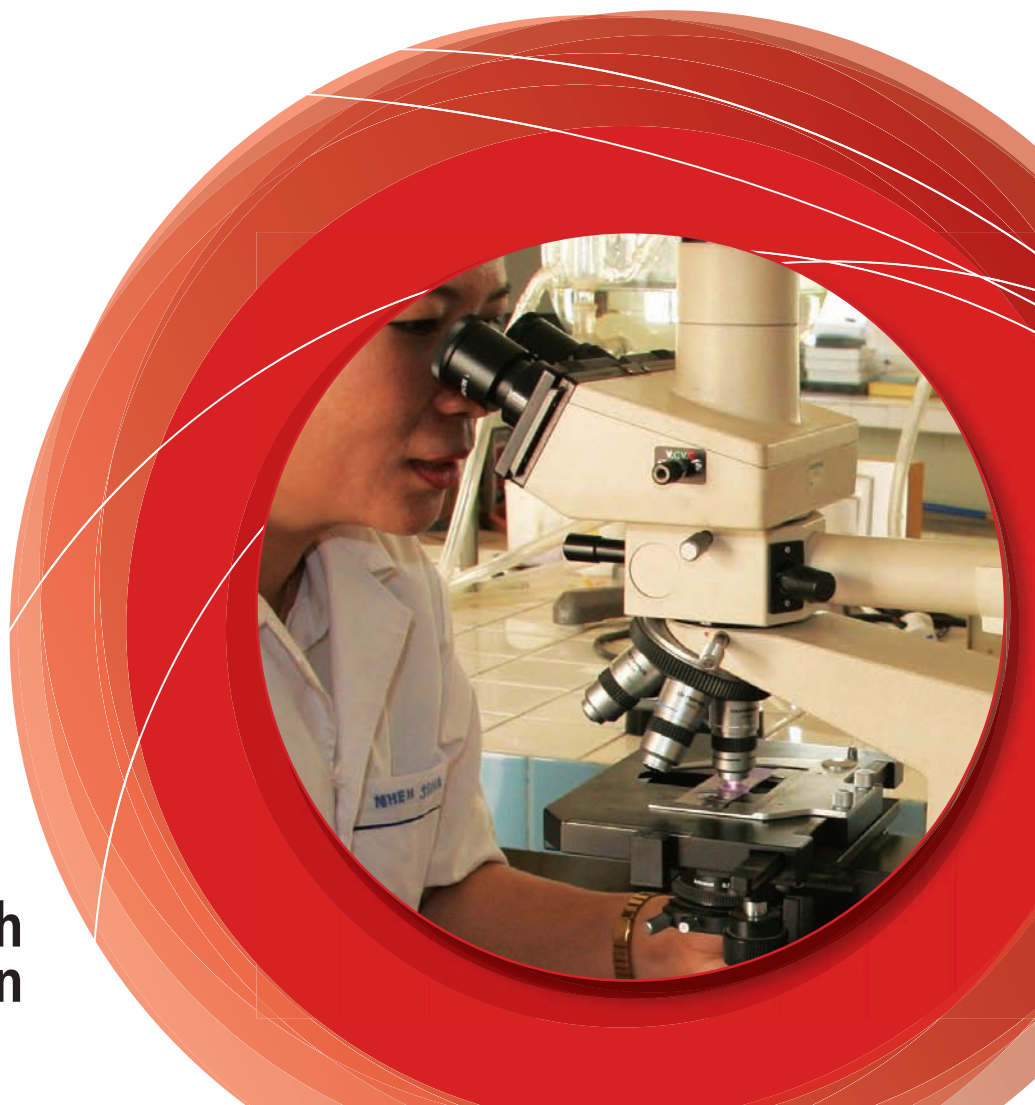


# Informal consultation on quality control of malaria microscopy

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WHO HEADQUARTERS, GENEVA, 3 MARCH 2006

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## **1. Introduction**

Parasite-based diagnosis is an important part of the case management of malaria, and WHO recommends that the demonstration of parasites should form the basis for treating malaria in all cases except among young children in areas of very high endemicity and during the control phase of malaria epidemics and emergencies (WHO 2004; WHO 2005b; WHO 2006). While rapid diagnostic tests are increasingly being used, microscopy-based diagnosis remains of central importance for species differentiation, parasite quantitation, management of severe disease and, for reasons of cost and sustainability, it is particularly important in areas with high case-loads.

It is also important for investigating treatment failures and for malaria surveys, clinical trials and monitoring the quality of other diagnostic approaches. Additionally, it has application in the diagnosis of many non-malarial febrile illnesses. Maintaining a proper setting and standards of competence are vital parts of a malaria microscopy programme because its performance is highly dependent on the competence of the technician. Programmes that aim to improve the quality of microscopy are under way in several countries, such as Cambodia, Indonesia, Oman, Sudan and the Solomon Islands, and are being supported by WHO's regional offices, Médecins Sans Frontières (MSF) and other agencies. Where possible, it is desirable that these programmes use common standards to define competence through microscopy quality assurance systems that are compatible with one another.

In April 2005 WHO's Regional Office for South-East Asia and the Regional Office for the Western Pacific held a biregional workshop on quality assurance for malaria microscopy in Kuala Lumpur. The workshop was held to review various issues concerning microscopy quality assurance (WHO 2005a).

One of the important recommendations made by the members of the workshop was that standardized methods for accreditation of competence in malaria microscopy should be established and these should be developed by WHO after additional expert consultation and consensus building. The Kuala Lumpur workshop also recommended that WHO, in collaboration with other agencies, should develop clear guidelines for quality assurance programmes for malaria microscopy. It also recommended that specific attention should be paid to reaching consensus on sample-size determination and the development of systems for the cross-checking routinely collected slides – that is, the development of scientifically sound methods of slide validation.

In addition it was recommended that WHO's basic malaria microscopy training manuals (WHO 1991a; WHO 1991b) and WHO's malaria microscopy bench aids (WHO 2000) should be reviewed and updated, with field-testing of draft materials before finalization. The workshop also recommended that electronic slide banks should

be developed as additional materials to be used in training and for improving competence and that these should be easily available through WHO.

To implement the recommendations of the workshop, WHO convened a 1-day consultation on microscopy accreditation, slide validation methods and training materials. A subgroup of participants who attended the Kuala Lumpur workshop and other key experts in the field convened on 3 March 2006 in Geneva to provide guidance on unresolved issues. The findings and recommendations detailed here will be circulated to a wider expert group for review and finalization; this circulation will include all who participated in the Kuala Lumpur workshop. These recommendations will guide WHO's activities in improving the quality of microscopy-based malaria diagnosis.

## **2. Aims of the consultation**

The informal consultation aimed to:

- review recent experiences of assessment schemes for malaria microscopy and propose standards of and methods for assessing the competence of expert microscopists at national level;
- review alternative slide validation schemes adopted by agencies and national microscopy quality assurance programmes and propose statistically sound methods for selecting and verifying slides;
- review the requirements for malaria microscopy training materials.

## **3. Accreditation of competence for malaria microscopists**

### **Kuala Lumpur recommendations**

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The Kuala Lumpur workshop recommended that WHO should set internationally recognized standards for competence in malaria microscopy both for senior technicians at the national level and for other institutions and bodies requiring that competence be demonstrated. Assessment and accreditation programmes

could be implemented by a variety of organizations all of which could use the same standards. Assessment and accreditation offer opportunities for self-improvement and potentially the development of career paths for top-level microscopists, as well as skills recognition by peers, trainees and public health programmes (such as malaria control and laboratory services); additionally, these programmes also offer the opportunity for international recognition of competence, which could facilitate regional and intercountry training. Discussions in Geneva centred on determining the methods of and standards for the assessment and accreditation of high-level microscopists; discussions also confirmed the consensus reached at Kuala Lumpur that the assessment and accreditation of microscopists in the general health services was the responsibility of national programmes and should be organized to take account of local conditions. The meeting recommended that WHO develop recommendations for national quality assurance programmes to inform this process.

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### 3.1 Recent experiences

Since the Kuala Lumpur consultation, a course on malaria microscopy assessment has been implemented in three countries (Cambodia, Indonesia and the Solomon Islands) by the Asian Collaborative Training Network for Malaria (ACTMalaria) with the support of the WHO Western Pacific Region–South-East Asia Region biregional programme for quality assurance for malaria microscopy. The course was developed by WHO in collaboration with the Philippines Department of Health and the University of the Philippines. Further courses are planned for the Lao People’s Democratic Republic and Myanmar, and an additional 7 or 8 courses will be held over the following 12 months. Accreditation of participants has been deferred while consensus on international standards is awaited.

The ACTMalaria courses run for 5 days and focus on developing competence in blood-film preparation and interpretation, staining and microscope use.<sup>1</sup> Top-level national microscopists enrol, and the course assumes considerable pre-existing competence and experience. The course does not include training in teaching or the management of quality assurance programmes. All courses have demonstrated a significant improvement in interpretation skills, using pre-course and post-course assessments. Experience in four countries indicates that achievement standards of 90% of species identified correctly and 50% of parasite densities (within  $\pm 25\%$  of true parasite density counted against white cells) identified correctly for a minimum of 20 blood films are realistic targets for top-level expert accreditation for national core-group microscopists.

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<sup>1</sup> Information on the ACTMalaria programme was presented by Ken Lilley. See the List of participants for additional information.

The planned microscopy training course in WHO's Eastern Mediterranean Region is expected to commence during the first quarter of 2007, and the draft curriculum was presented at the Geneva consultation. Developed by the Government of Oman and WHO's Regional Office for the Eastern Mediterranean, with support from external consultants, this course also targets senior microscopy technicians.<sup>2</sup> This course lasts 4 weeks and the curriculum aims to increase competence in malaria microscopy and to develop training skills (using a "training of trainers" scheme), skills in managing national training courses, supervision, and the setting up of quality assurance programmes as well as the development of slide banks. The Medical Technology Academia of Oman will be approached to grant academic recognition for the course and to participate in monitoring and accreditation activities. Although the course has more extensive aims than the ACTMalaria course, the assessment of competence in blood-film interpretation could apply common criteria for assessment. The plan for accreditation of diagnostic accuracy requires that > 90% of test slides are interpreted correctly in order to achieve the top level (designated as a "core trainer") in addition to a score of > 90% in other aspects, such as film preparation, training and the management of quality assurance programmes. Participants who achieve > 90% in all aspects will be recognized as "national validators".

MSF's malaria laboratory quality assurance programme assesses performance in laboratories in Africa, Asia and the Americas and is supported by this Agency.<sup>3</sup> The microscopy training centre in Nairobi, Kenya, is equipped with 15 microscopes intended for use in training and competency programmes. Experience has shown the importance of including assessments of staining, film preparation and the recognition of artefacts in blood films when overall competency is assessed. Additionally, the importance of adjusting the slide sets to reflect the parasites most likely to be encountered by local microscopists (for example, *Plasmodium falciparum* in much of Africa) has been recognized, although this should not compromise the advantages of adopting international standards for competency.

MSF's experience indicates that 100% sensitivity can be expected for ++, +++ and ++++ thick blood films, and > 95% specificity is realistic when equipment is good and a laboratory is well managed. Low levels of parasitaemia are more problematic, and MSF has adopted a goal of > 90% correct for 1–4 trophozoites/100 fields and > 95% correct for 5–9 trophozoites/100 fields. This is now being field-tested. These performance standards are considered realistic with good training, microscopes, staining and correct workload management. Lower levels of performance occur when

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<sup>2</sup> Information on the course developed by the Government of Oman and WHO's Regional Office for the Eastern Mediterranean was presented by Majed S. al-Zedjali from Oman's Ministry of Health. See the List of participants for additional information.

<sup>3</sup> Information on the MSF programme was presented by Derryck Klarkowski of Médecins Sans Frontières–Holland. See the List of participants for additional information.

working conditions are not optimal. Performance above the set thresholds should be expected from top-level microscopists in a competency assessment programme. Issues related to blood film preparation should be addressed by providing good standard operating procedures; although WHO does not provide standard operating procedures in its training materials, MSF uses standard procedures that have been tested through experience in its laboratory network.

A performance assessment scheme for malaria microscopy was introduced to the external quality assurance network in Africa after the Kuala Lumpur workshop; it is coordinated by the WHO Lyon Office for National Epidemic Preparedness, the Regional Office for Africa and the National Institute for Communicable Diseases in South Africa.<sup>4</sup> For the first survey a mass-produced panel of six slides and an accompanying questionnaire were sent to the 72 national public health laboratories participating in the network. The results indicated there was a need for more intensive assessment and training and that there was a lack of essential good quality equipment at many sites.

### 3.2 Findings and recommendations

To be competent in malaria microscopy technicians must have adequate skills in preparing, staining, reading and interpreting blood films. Overall performance is influenced not only by the microscopist's competence but also by other factors, including working conditions, the number of slides that need to be read and equipment (WHO 2005a). Accreditation of competency should form part of the process of improving diagnostic performance and should be standardized internationally to improve the skills of the national core group of expert microscopists as much as possible.

Training to improve competency at the national core-group level may include training in programme management, education methods (such as the "training of trainers"), blood film preparation and staining, and slide interpretation. Assessments of competency may include assessment of all these abilities, depending on the aims of the specific programme. It is appropriate to have an internationally approved and standardized method to assess and accredit competence in interpreting blood films, as was recommended by the Kuala Lumpur workshop, to serve as a stand-alone certification scheme or to form part of a wider programme of competency assessment (as in the example from Oman above).

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<sup>4</sup> Information on this scheme was presented by Leigh Dini, National Institute for Communicable Diseases, South Africa, and Jean-Bosco Ndiokubwayo, WHO Regional Office for Africa, Communicable Diseases Surveillance and Response Unit.

### 3.2.1 Components of competency review

The following components of competency review were recommended as the basis of a WHO-coordinated international accreditation scheme for competency in blood film reading and interpretation.

### 3.2.2 Aim of accreditation

The accreditation of competency should be standardized for senior malaria microscopists.<sup>5</sup> Although these standards are primarily intended for senior national validators and trainers, the same standards may also be applicable to other settings, such as nongovernmental organizations and the private sector.

### 3.2.3 Principles on which assessment should be based

The following principles should form the basis of assessments.

- Grading must be realistic, achievable and sustainable. A planned re-assessment of standards should take place after the programme commences and experience has been accumulated.
- Competency assessment must include an evaluation of the sensitivity of reading and identification of malaria species as well as quantification of parasite density (based on counting, not estimation).<sup>6</sup>

### 3.2.4 Minimum standards for programme structure

Criteria that outline the minimum standards for training programmes.

- Programmes must last for a minimum of 5 days.
- At a minimum they must include:
  - ~ preparing blood films, including safe blood handling,
  - ~ staining blood films,
  - ~ setting up and using a microscope,
  - ~ reading and interpreting blood films.
- Training should include one-to-one interaction between facilitators and participants; thus a maximum 12 participants is appropriate.

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<sup>5</sup> This implies that there should be entry criteria for courses in terms of experience. Countries should set standards to ensure that participants have a certain number of years' experience before they enrol on the course; these standards must reflect local workload and capacity.

<sup>6</sup> There was no consensus on whether assessment should include blood-film assessment and staining, although there was consensus that these should be taught in all competency courses that are linked with assessment.

- The programme should include a pre-test at commencement of the course (with immediate feedback) and a blinded post-test assessment (a “final competency assessment”) on which accreditation should be based.
- The facilitator should be a highly experienced microscopist and trainer.
- Competency accreditation should be standardized using well validated blood films rather than relying on the facilitator’s judgement (see Boxes 1 & 2).

### BOX 1 Minimum slide set

The minimum slide set should include 20 slides<sup>a,b</sup>:

- 5 negative slides
- 1 slide of an unusual presentation
- 2 slides of *P. falciparum* with a density of 50–100 parasites/mm<sup>3</sup>
- 3–5 *P. falciparum* slides with a density of 300–800 parasites/mm<sup>3</sup>
- 2–4 *P. vivax* slides (2 at a density of 100–500 parasites/mm<sup>3</sup> and the remainder at > 500 parasites/mm<sup>3</sup>)
- 2 *P. malariae* slides at densities of > 500 parasites/mm<sup>3</sup>
- 2 *P. ovale* slides at densities of > 500 parasites/mm<sup>3</sup>
- 1 slide with mixed species

Morphological changes of parasites attributed to the effects of antimalarial drugs should be covered in training but not in the examination.

### BOX 2 Conditions for final assessment (accreditation)

- Assess competency in species identification and parasite density (counting parasites against white blood cells)
- Give participants 10 minutes per slide
- Spread the assessment over 2 days
- Allow normal materials used in the laboratory to be used during examination (for example, WHO’s *Bench aids for the diagnosis of malaria infections*)
- Ensure high-quality binocular microscopes with electric light are provided
- Present slides in random order
- Maintain strict examination conditions (ensuring quiet and confidentiality)

<sup>a</sup> Each slide should include a thick film and a thin film; to reflect normal working conditions, microscopists should be allowed to read either or both. To implement this programme, standard operating procedures for producing slide sets must be provided and existing slide sets should be expanded or new sets developed.

<sup>b</sup> The number of *P. falciparum* and *P. vivax* slides should be adjusted to reflect local prevalence.

### 3.2.5 Minimum standards for final competency assessment

During the meeting and in subsequent correspondence there was considerable discussion on the appropriate standards for the final assessment of competency and the composition of the slide set used for assessment. A slide set to be developed over the following months by the Asian Collaborative Training Network for Malaria (ACTMalaria) with the support of the WHO Western Pacific Region–South-East Asia Region biregional programme will be used for assessments during a 12-month period and, when appropriate, an accreditation certificate will be awarded based on the interim criteria detailed below. A meeting will be held after the 12-month period to assess the results of the slide set and demonstrated competence. Criteria will be revised at that time based on the evidence and experience.

- *Slide set:* 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 blood films, validated by multiple expert microscopists who participate in external quality assurance programmes; films should be checked for species identification by polymerase chain reaction (PCR).<sup>7</sup>
- *Assessment conditions:* a relaxed atmosphere is important, and participants should have the opportunity to familiarize themselves with the environment and equipment prior to the assessment (that is, during the preceding workshop).<sup>8</sup>
- *Rating:* the recommendation is that accreditation should last a maximum of three years initially. Earlier reassessment should be available for those who are not performing well. Accreditation should be done independently of external quality assurance and proficiency-testing programmes, in which all microscopists and laboratories should be enrolled (when programmes are available). Table 1 outlines the standards that will be used as an interim measure and reassessed after one year; these ratings are based on experience with assessments in Asia that were presented to the meeting.

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<sup>7</sup> The slide set for assessments includes only two slides with a low density of parasites. Detection of low densities, while important in microscopy competence, is also influenced significantly by chance because of the low number of fields containing parasites, so it is not appropriate to use this as a basis for accreditation. Measuring accuracy in counting parasites at higher densities indirectly assesses the same skills of parasite recognition and is less influenced by chance.

<sup>8</sup> Written and pictorial reference materials are 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 aids for the diagnosis of malaria infections* and be free to bring in their own references. However, it is advisable for these to be checked for accuracy by the assessor. Although the decision on whether to allow participants to use reference materials was the subject of some disagreement at the 2006 consultation, a clear majority of experts present considered that they should be available.

**Table 1. Interim grades for final competency assessment for expert accreditation<sup>a</sup>**

Grade	Accuracy of species identification <sup>b</sup>	Parasite quantitation <sup>c</sup>
1. Expert	≥ 90%	≥ 50%
2. Reference	≥ 80%	≥ 40%
3. Advanced	≥ 70%	≥ 30%
4. In training	< 70%	< 30%

<sup>a</sup> Both identification and quantitation criteria must be met. Additionally, certificates should include the number (1–4) and the percentage range of each grade to allow comparisons to be made across languages and nomenclatures. (Some experts participating in the 2006 meeting preferred the terms “Practising”, “Accredited” or “Competent” for Grade 3.)

<sup>b</sup> Some experts believed that these pass marks should be higher. Standards should be reviewed after implementation in light of the results obtained.

<sup>c</sup> Percentage of quantitation within 25% of true count.

### 3.2.6 Lessons from current courses

The handling of the results from course assessments is sometimes a sensitive issue because participants often hold senior positions in ministries of health or academic institutions, and their performance may have implications for their professional relationships and even their employment. Although the results of the ACTMalaria assessments had previously been handled confidentially, in recent courses rankings were openly discussed with the participating group. If accreditation certificates are awarded then results will be publicly disclosed and, for this reason, courses should concentrate on helping participants improve their skills rather than focusing only on assessing competence. Additionally, opportunities and materials should be made available for self-learning between courses and assessments, and measures should be taken to reduce the impact of a temporary reduction in performance (for example, due to stress or illness). The final assessment in the ACTMalaria course is carried out over 2–3 days.

Experience acquired from the implementation of previous courses indicates that the following are important.

- One-to-one interaction between the facilitator and participants is essential. The number of participants must be limited (for example, to 12 in the ACTMalaria courses), and the courses should last a minimum of 5 to allow for sufficient interaction and assessment and development of skills.

- The facilitator must be highly experienced and have proven competency. The facilitator must also have the ability to create a relaxed and respectful atmosphere. A pool of such trainers is needed.
- The curriculum must be flexible enough to accommodate local needs and skills.
- Parasites in the majority of slides used for training and assessment must reflect locally prevalent species.
- Blood films must be well validated. Additionally, the assessment procedure (and the course facilitator) must be flexible enough to account for errors in previously validated films. Slides for species identification that come from slide banks should preferably have had the species confirmed by PCR. A number of internationally validated films used in some assessments were found to have mixed infections, demonstrating the importance of PCR screening for all slides in slide banks.
- Assessments conditions should be similar to those normally experienced by the microscopists, for example, there should be access to bench aids and other materials to support parasite identification.

### 3.2.7 Slide banks

The Kuala Lumpur workshop discussed a number of models for slide banks that could be used in assessment programmes; participants also discussed the resources required to establish and maintain them. Validated sets of malaria blood films, which are necessary for standardizing assessment and for training, exist in various countries and are available from the Malaria Research and Reference Reagent Resource Center of the United States National Institute of Allergy and Infectious Diseases. These were developed by WHO and are used for proficiency-testing schemes, such as those of the United Kingdom's National External Quality Assessment Service, but additional resources are needed before slide banks with slides validated for malaria microscopy quality assurance programmes will be more widely available. There is a role for WHO in facilitating this. The standard operating procedures for preparing slides and developing slide banks need to be reviewed with the objective of reaching consensus based on the procedures that have been adopted by MSF, the Malaria Research and Reference Reagent Resource Center, Hydax Inc., the Naval Medical Research Unit 2 and others.

## 4. Validation of malaria slide results in quality assurance programmes

### Kuala Lumpur recommendations

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All national malaria control programmes promoting microscopy for diagnosis should have a well structured quality assurance programme. All laboratories and technicians regularly diagnosing malaria by microscopy should be enrolled in a quality assurance programme.

Traditional quality assurance programmes for microscopy emphasize validation by having expert microscopists re-check slide results to monitor the performance of technicians. Old practices of checking all slides with positive results and 10% of those with negative results have become unsustainable due to the number of slides involved. The Kuala Lumpur workshop recommended that national quality assurance programmes for malaria microscopy should focus on regular retraining and competency assessment and that a sustainable form of slide validation should also remain a part of quality assurance programmes. Although competency testing forms the main method of assessing the expertise of technicians, the validation of slides read in the field allows for monitoring of performance over time and also allows additional problems to be detected, such as poor staining, poor quality of slide preparation, the inadequacy of supplies and equipment, and other factors affecting performance in malaria microscopy (WHO 2005a)

Slide validation schemes must be restricted to reading a limited number of slides – and acting upon the results – with the resources available. Slides must be selected and the sample size calculated to ensure that the results of validation are meaningful for assessing the performance of malaria microscopists. The experience in the Philippines, which used an alternative method based on the selection of a small sample using lot quality-assurance sampling (LQAS), as well as alternative methods that considered both statistical and operational implications were reviewed at the meeting to identify potential slide validation schemes.

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## 4.1 Recent experiences

Two validation programmes currently in use were discussed: the system adopted by MSF laboratories in Africa, Asia and the Americas and the scheme implemented by the Department of Health in the Philippines.<sup>9</sup> Other experiences were also discussed at the 2006 consultation.

The MSF system is based on the proportion of agreement between the first reading and expert re-examination of 120 slides per microscopist per year – that is, 10 slides per month. The 10 selected slides are read monthly and the results analysed in 4-monthly cohorts of 40 slides; batches of 40 slides (10 slides per month) were considered to be the minimum sample size that could be used for meaningful analysis. Examining 10 slides each month allows major problems of poor performance to be detected. Using a larger sample of 60 slides (collected over 6 months), or 120 slides (collected over 12 months), would be statistically more rigorous but it has the disadvantage of offering feedback less frequently. Using a minimum number of slides for quality control is intended to ensure that validators can cope with the workload. Slide selection is specified as 5 low-density positive slides (+ and ++) and 5 negative slides per month. The 5 low-density positive slides and the 5 negative slides should be selected randomly from register entries. If slides were selected randomly from all slides (irrespective of positivity), it is likely there would be too few positive or negative slides (depending on prevalence) to produce a meaningful assessment of false-positive and false-negative rates. Poor performance is addressed using a variety of strategies, such as retraining and programme management (for example, by addressing workload and equipment issues), depending on the type of problem identified.

Drawbacks of the MSF system include the potential for validators to be influenced by the knowledge that they will be cross-checking an equal proportion of positive and negative slides. This is unlikely to be a significant problem because, although there are equal numbers of positive and negative slides as reported by the laboratory, these slides may well contain errors and the true proportion of positive and negative slides may well be different. Obviously, the slides should not be marked as positive or negative so the validator is blinded to the recorded result. There is also the possibility that field microscopists might select slides with results of which they are sure; however, this is a problem that is common to all quality control programmes that use cross-checking and requires systematic management. Although it is possible that the selection of slides sent to the validators may be biased, this cannot be avoided in any system without incurring considerable expense. But even if selection is biased, it is likely that major deficiencies in competence, and problems affecting performance, will still be detected. However,

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<sup>9</sup> Information on MSF's system was presented Derryck Klarkowski of Médecins Sans Frontières–Holland. Information on the system used in the Philippines was presented by Jenny Luchavez of the Philippines' Research Institute of Tropical Medicine.

this has to be seen as a potential limitation of any quality assurance programme. MSF will soon be introducing an additional standardized slide-preparation and staining component to the quality control assessment.

The system adopted in the Philippines is described in detail in the report from the Kuala Lumpur workshop (WHO 2005a). In brief, it assumes that 10% of slides will be positive and aims to discover failures in parasite detection that fall below 80% sensitivity and 100% specificity with 95% confidence intervals; under the validation programme a total of 96–103 (around 100) randomly selected slides should be re-checked annually if the microscopist sees < 1000 negative slides per year, or 120 should be re-checked if the microscopist sees 1001–5000 negative slides per year. This system uses a sample size calculated similarly to that of the LQAS tables used for tuberculosis microscopy (APHL 2002); it is calculated using positivity rates, allowable error and workload. The resulting selected number of slides to be cross-checked, based on estimated prevalence, is similar to those selected by MSF (100–120 per year). Since slides are selected randomly in the Philippines, most are expected to be negative. In practice, reductions in the incidence of malaria in the Philippines after improvements in case management and vector control, have resulted in many microscopists failing to see a sufficient number of cases to fulfil the quota of slides for cross-checking. The quality of slide staining is also included in the evaluation (weighted to 25% of the final result).

A slide selection method based on the LQAS allows a small number of slides to be selected for quality control with a sound statistical basis for interpreting the results. However, the method used to select the sample size, which depends on prevalence and workload, is relatively complicated and not necessarily clear to all participants (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 the LQAS has been endorsed as an appropriate method of quality control. Test centres pass or fail depending upon whether they are below or above the stipulated number of acceptable errors. While this allows for good detection of 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, and this may reduce the analytical value. This combination of factors may account for the low discrepancy rate (0–3%) reported by the validation programme in the Philippines.

In summary, both models select a similar number of slides. Although the MSF model involves less randomization in slide selection it allows medium-level performers to be identified (rather than categorizing them as pass or fail). The MSF model also potentially includes more analytically useful low-density positive slides, which are likely to delineate performance levels, and it also operates independently of seasonal or geographical fluctuations in prevalence.

## 4.2 Other statistical issues<sup>10</sup>

The reporting of the results of cross-checked slides must be consistent to avoid misunderstanding between validators and those whose performance is being checked. The main areas to be considered are listed below.

- The concepts of “sensitivity” and “specificity” are generally well understood but are not readily applicable to non-randomly selected slides (such as the MSF method) or to sets with very a low prevalence of positive results (the Philippines method). In these contexts it would be more appropriate to use the proportion of positive slides correctly identified and the proportion of negative slides correctly identified. The possibility of chance variation leading to the validator missing parasites on a weak-positive film also has to be built into the way quality control is reported, and there should be more tolerance for error in low-density films than in high-density films.
- The “false-positive rate” is important in assessing performance but it may be measured against either the total number of cases (slides) or against the number of true-negative slides (preferably a consistent number). Using a consistent number of true-negative slides gives a better indication of the microscopist’s performance because it operates independently of prevalence. As above, tolerance must take into account the element of chance involved in seeing parasites in very low-density films.
- The “false-negative rate” is important clinically but it is subject to chance when there are very low densities of parasites.
- “Proportional agreement”  $[(\text{true positive} + \text{true negative})/\text{total}]$  gives a single number to indicate screening performance. This may be the weighted average of the sensitivity and specificity or the weighted average of the positive predictive value and negative predictive value. If the same number of negative slides and positive slides are selected (as in the MSF method) then this number is a simple average (rather than a weighted average) of the positive predictive value and negative predictive value, so the value is comparable between centres. Using this measure implies that it is considered as bad to misdiagnose someone who truly has malaria as it is to misdiagnose someone who truly does not have malaria.
- The “positive predictive value” (that is, the probability that those who are diagnosed by microscopy as having malaria truly do have the disease) and the “negative predictive value” (that is, the probability that those who are found by microscopy not to have the disease truly do not have it) are clinically useful for field studies but they vary with the prevalence of malaria. By selecting predetermined numbers of negative slides and positive slides, unbiased estimates

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<sup>10</sup> An overview of statistical issues was presented by Hilary Watt, London School of Hygiene and Tropical Medicine. See the List of participants for additional information.

of true positives and true negatives can be obtained, but when small numbers of slides are involved, as in the MSF method, the description as false-negatives and false-positives will provide clearer information to the technician who is being assessed.

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. Determining this threshold requires benchmarking – that is, determining what constitutes a reasonably achievable standard by comparing many results and then selecting the outliers and providing remedial help for them (by addressing competency, deficits in materials and/or workplace conditions). The threshold could be determined as a variation from the mean performance (for example, 2 standard deviations) or performance well below that which is considered acceptable competence for clinical reasons. The system adopted by MSF and described above provides a representative sample for performing a slide-validation scheme. MSF laboratories achieved a mean performance of 90% of slides interpreted correctly. More information on laboratory performance will be collected during 2006 to allow evidence-based decisions to be made on appropriate standards.

### 4.3 Findings and recommendations

Some method of validating or cross-checking performance is necessary, though this should be seen as supplementary to a programme of competency assessment and retraining. Cross-checking at a central validation centre (as in the minimum standard recommended above) may be replaced by regular supervisory visits (conducted at least every 2 months) that include cross-checking at the technician's workplace. This is preferable but requires significant resources.

The following list details the proposed recommendations for minimum acceptable standards of slide validation for quality assurance in malaria microscopy. Validators should be subject to a regular external competency assessment to maintain their skills and credibility.

- Validation must always be coupled with a system for assessment and correction of poor performance that is the result of:
  - ~ technical incompetence,
  - ~ problems in the workload,

- ~ problems in the work environment (and perceived pressure to produce certain results),
- ~ problems with reagents,
- ~ the quality of microscopes,
- ~ the lack of a support network,
- ~ lack of supervision, or
- ~ non-work-related factors.

All of the above factors must be considered as potentially responsible for poor performance. Staff competence may be a minor component of poor quality: good staff cannot perform optimally with poor infrastructure.

- Workplace assessment should include an evaluation of blood-safety measures.
- Feedback should be timely, given to all participants and include ratings of individual performances or the test centre's performance against the benchmark average or acceptable performance.
- In terms of slide selection the following should be considered:
  - ~ a minimum of 10 slides a month should be selected for quality control (equivalent to 120 slides per year); these should be read monthly but analysed in batches (for example, batches of 40);
  - ~ more slides can be cross-checked as long as the quality of cross-checking is not compromised;
  - ~ positive and negative slides should be randomly selected from the laboratory register so that half have positive results and half have negative results;<sup>11</sup>
  - ~ where possible, slides should be selected randomly from a list of positive results and negative results by an independent member of the lab or the validation laboratory;
  - ~ positive slides with lower parasite densities should be selected;
  - ~ validators should be blinded to results of field microscopist.
- Performance should be reported as:
  - ~ proportional agreement (that is, the percentage of positive slides correctly identified and the percentage of negative slides correctly identified, which is represented as (true positive + true negative)/total, and
  - ~ the false-positive rate (as a proportion of true positives), then
  - ~ the false-negative rate (as proportion of true negatives);

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<sup>11</sup> If insufficient positive or negative slides are present during a month, then the number should be made up to 10 by increasing the number of negative (or positive) slides; however, the effect of this should be borne in mind when assessing the results of validation.

- ~ the idea of agreement should include the correct identification of parasite species;
- ~ the allowable false-positive rate should be low or zero;<sup>12</sup>
- ~ when the number of slides examined by the laboratory is low and inadequate to meet the requirements of the proposed slide validation scheme (randomly selected 5 positive and 5 negative slides per month) the threshold for supervisory visits needs to be adjusted. In this situation, the parasite density should be taken into account in addition to the number of positive and negative slides. For example, missing the only positive slide, when this is of low parasite density, is less serious than missing 2 of 5 positive slides if all are of high parasite density.
- Recommended thresholds for action should be determined after review of the experience in implementing the proposed slide validation scheme. Criteria may vary for different settings.

Validation plans can be structured around either regular supervision visits (for example, every 2 months) or regular performance assessment programmes. Some programmes may have the resources needed to conduct more frequent assessments. The number of slides a validator can reliably read in a day (about 6–8 per hour) should be taken into account when planning performance assessment activities.

## 5. Revising WHO training manuals and bench aids on malaria microscopy

A consultant has been engaged by WHO to revise the existing manuals, field-test the revisions and consult widely on the revisions among experts in malaria microscopy. The basis for revising the training manuals was presented and discussed at the consultation and recommendations were made for additions and improvements.<sup>13</sup>

### 5.1 Learner's guide

The revisions being made to the manual for trainees are designed around competency-based training and will concentrate particularly on:

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<sup>12</sup> When setting the allowable false-positive rate the prevalent parasite densities should be taken into account. Where these are commonly low it is more likely that false positives are low densities of parasites detected by the field microscopist but missed by chance by the validator. Where parasite densities are high, a false positive rate of 0 may be appropriate.

<sup>13</sup> Information on the revisions was presented by John Storey, consultant. See the List of participants for additional information.

- ensuring the manuals are sufficient to stand alone as training manuals for basic microscopists with no previous training and that they are based on a 5-week curriculum (or 2-week syllabus for medical technologists requiring refresher training);
- ensuring text is simple enough to make translation easy and for those whose first language is not the language of the training manual;
- describing better the educational aspects of competency-based training (tutor's guide);
- improving the quality of multiple-choice questions (tutor's guide);
- reviewing the adequacy of illustrations;
- ensuring there are appropriate cross-references to other training material;
- increasing the level of detail on parasite density calculation.

The plus system should be retained as a method suited to busy field situations but the method for quantifying parasite density as +, ++ or +++ should be standardized. Additionally, the limitations of this system should be clearly defined. Emphasis should be placed on counting parasites against white blood cells where possible to obtain accurate counts.<sup>14</sup>

It is recommended that an annex be added that contains detailed standard operating procedures for the preparation of blood films and the preparation and use of stains;<sup>15</sup> also, standard micro-photographs of correctly stained blood films should be included. Standard operating procedures for the preparation of blood films and the preparation and use of stains are under review as part of WHO's development of recommendations for slide banks.

The existing version of the manual uses colour drawings of malaria parasites rather than photographs. Many trainees appear to prefer these to the photomicrographs found in the second edition of *Bench aids for the diagnosis of malaria infections* but it is not yet clear which drawings or photographs are most appropriate for training. The consensus is that both are useful, and drawings could be retained in the training manuals with photomicrographs included either in the manuals or as supplementary material (for example, in WHO's book on bench aids). Any additional graphics to be included in the revised version will be determined after further consultation. References to suitable photographic libraries and training materials in electronic format will also be included.

## **5.2 Tutor's guide**

Sections will be added to explain the concept of competency-based learning, as will an overview of ethical issues in malaria microscopy. The new manual will emphasize the place of slide banks in microscopy training. Consideration will be given to developing an accompanying CD-ROM on teaching methods.

## **5.3 New manual on implementing national quality assurance programmes**

The Kuala Lumpur workshop recommended that WHO produce recommendations on developing national quality assurance programmes for malaria microscopy, including methods for accreditation and slide validation as described above. These recommendations will be published as a third manual in the series. It is not an option to include this material as an annex to a training manual because there would be problems with the size of the publication and the content may be relevant to different personnel than those who use the training manual.

## **5.4 Bench aids**

The photomicrographs in the current bench aids publication are considered to be high quality, although the text would benefit from minor revisions and the font is considered too small by some workers. Putting the material onto a CD-ROM may improve access, although the loss of control over the quality of subsequent printing may affect the quality of the product.

## **5.5 Accompanying material**

WHO is investigating the use of CD-ROMs and collaboration in further development of CD-ROM-based training materials. When finalized, references will be included in the training manuals.

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