A WHO external quality assurance scheme for malaria nucleic acid amplification testing

8–9 June 2015, London, United Kingdom
Meeting report
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1. BACKGROUND

Since 2010, WHO has recommended that every case of suspected malaria be confirmed by a diagnostic test. In 2012, the T3 (Test, Treat, Track) Initiative was launched to encourage malaria-endemic countries, donors and the global malaria community to increase diagnostic testing, treatment and surveillance for malaria. Since then, malaria diagnosis has become an essential component of malaria control strategies. The pivotal role of diagnostic testing for malaria is reinforced by the Global Technical Strategy 2016–2030, adopted at the Sixty-eighth World Health Assembly (A68/28), for universal access to malaria prevention, diagnosis and treatment and accelerated efforts towards elimination. As part of this strategy, new tools and approaches are expected to become available to target the infectious parasite reservoir in humans.

While quality-assured rapid diagnostic tests (RDTs) and microscopy are the primary diagnostic tools for confirmation and management of cases of suspected clinical malaria, their sensitivity is too limited for efficient detection of low-density parasitaemia or sub-microscopic infections. These infections are common in both low- and high-transmission settings and contribute significantly to the residual, continuous transmission of malaria in low-transmission settings. Many nucleic acid amplification tests (NAATs) are available, which provide better diagnostic performance but imperfect reproducibility as compared with RDTs or microscopy for the detection of low-density parasitaemia and uneven reproducibility.

On the basis of the recommendations of the Malaria Policy Advisory Committee in March 2014, WHO issued a policy brief on malaria diagnostics in low-transmission settings (WHO/HTM/GMP/2014.7), recommending that “use of NAA methods in malaria programmes should be considered for epidemiological research and surveys to map sub-microscopic infections in low-transmission areas”. In view of the potential role of NAATs in elimination settings, the document indicated that “an international external quality assurance system is strongly recommended to ensure that data obtained from NAA assays are reliable and comparable”.

As part of WHO’s work in promoting access to quality-assured malaria diagnostic tests, the Global Malaria Programme organized a meeting on preparation of an external quality assurance (EQA) scheme for malaria NAAT on 8–9 June 2015, in London, England, which was attended by 20 participants, including experts in malaria molecular-based testing, representatives of reference laboratories, institutions involved in the quality assurance of diagnostic tests, research institutes and funding partners.

The main objectives of the meeting were to:

- define customer requirements for an NAAT EQA scheme,
- identify the EQA materials and panels needed for an NAAT EQA scheme,
- define the structure of the NAAT EQA scheme,
- identify strategies for resource mobilization and
- reach consensus on the next steps, roles and responsibilities.
2. EXPERIENCE WITH EXTERNAL QUALITY ASSURANCE FOR MALARIA NUCLEIC ACID AMPLIFICATION TESTING

2.1 Controlled Human Malaria Infection (CHMI) trials network

Several research groups routinely conduct trials of controlled human malaria infection (CHMI), usually to evaluate the efficacy of antimalarial drugs or vaccines. In controlled infection, malaria-naive volunteers are infected by mosquito bites or by injection of sporozoites, and the emergence of blood-stage parasites is carefully monitored in order to initiate treatment as early as possible to ensure the safety of the volunteers and avoid or minimize the appearance of symptoms. Efficacy and safety end-points are usually based on microscopy data; however, NAATs are increasingly being used, as they allow earlier detection of blood-stage parasites, thus ensuring participants’ safety and data resolution. Several trials have already been conducted exclusively with NAAT-based efficacy and safety end-points. The NAATs used in CHMI laboratories usually target conserved sequences of the 18S rRNA gene.

In order to evaluate the accuracy and comparability of NAATs, several laboratories conducting CHMI trials performed an EQA exercise. Six centres each received a blinded panel of 60 *P. falciparum* samples prepared from a synchronous, ring-stage, high-parasitaemia culture of the 3D7 strain diluted into type A+ whole human blood to concentrations of 300,000 parasites per mL (p/mL), 6000 p/mL, 600 p/mL, 60 p/mL, 6 p/mL and 0 p/mL (negative samples). Aliquots were prepared according to the procedures in place at each laboratory at volumes ranging from 50 µL to 500 µL before being frozen down and transported on dry ice. Each laboratory was given a minimum of 10 samples at each concentration and 10 negative samples, all blinded, for a total of 60 samples. The NAATs at each laboratory achieved a specificity of 100% and limits of detection between 600 and 60 p/mL. Except for the samples with very low parasitaemia, each laboratory found a sensitivity of 96.7–100%. Good agreement (0.5 log10 difference; < 10% CV) was found between measured and expected values, except in one laboratory, where a general quantitative shift was observed and subsequently resolved.

This small-scale pilot EQA exercise showed that the NAATs used in CHMI laboratories produce accurate, comparable data and demonstrated the applicability of an EQA scheme to evaluate the performance of multiple NAAT protocols. To formalize and extend this initial exercise, the laboratories involved suggested creation of the Molecular Malaria Quality Assessment (MolMalQA), a network of reference and partner laboratories to support establishment of an EQA scheme for malaria NAATs. In addition to the anticipated financial resources required, the challenges identified for the scheme include standardization of materials (because of the complexity of the *Plasmodium* life cycle) and accommodation of the variety of tests and source material in use, thus ensuring the compatibility of tests with liquid and dried blood spots and possibly RNA (in addition to DNA) extraction and amplification techniques.

2.2 United Kingdom National External Quality Assessment Service

The United Kingdom National External Quality Assessment Service (UK NEQAS) is a public service that provides EQA schemes for many diseases. According to its website, it comprises a network of 390 schemes operating in 26 centres based in large hospitals, research institutions and universities throughout the United Kingdom. The services cover qualitative and interpretative investigations in reproductive science, cellular pathology, clinical chemistry, genetics, haematology, immunology and microbiology. Each is directed by experts in the field, with support and advice from steering committees.
UK NEQAS Parasitology established a pre-pilot scheme for malaria NAATs in 2014 and presented the results of two pre-pilot with samples distributed to 28 registered malaria laboratories worldwide in which nested PCR or real-time PCR was used. Samples were prepared from infected donors and diluted in blood negative for *Plasmodium* species to concentrations of 20,000 p/mL, 5,000 p/mL, 1,000 p/mL and 0 p/mL (negative samples), divided into 0.5-mL aliquots and lyophilized. The first round included *P. falciparum* and *P. vivax* species and the second one *P. ovale* and *P. malariae*. Panels containing one sample at each concentration were shipped blind at ambient temperature and reconstituted on site with molecular-grade water. Results were returned from 24 and 25 laboratories during the first and second pre-pilot phases, respectively. Samples could not be shipped to some West African countries during the outbreak of Ebola virus disease, and customs procedures in Brazil and Peru were lengthy and costly. A few false-positive and false-negative results were reported in each study, possibly because of errors in data entry (Figs 1 and 2).
Although samples could be ranked by parasitaemia quantitatively, the absolute parasite densities reported were lower than expected. Lyophilization was found to be a straightforward means for reconstitution and DNA extraction and allows transport at ambient temperature. It was concluded that the pre-pilots rounds fulfilled the criteria of EQA, in that they helped participants take individual action to investigate and remedy any discrepant results.

Pilot rounds will be performed in August 2015 and February 2016, with 10 new participating laboratories and the possibility of web-based reporting of results. The full scheme is scheduled to start in April 2016, and an estimate of the costs should be available by September 2015.

2.3 Worldwide Antimalarial Resistance Network

The WorldWide Antimalarial Resistance Network (WWARN) is a global platform for international research on antimalarial drug resistance. Many groups and laboratories face the challenge of comparing data generated on different platforms, with different protocols in different places. WWARN therefore includes quality assurance programmes to improve data comparability and encourage adoption of standard procedures. The quality assurance and quality control programme, which complies with ISO 17043, is a proficiency-testing scheme for pharmacology laboratories involved in studies of the pharmacokinetics and pharmacodynamics of antimalarial agents, which has contributed to improving the performance of the laboratories. An EQA scheme has been set up for molecular typing of Plasmodium parasites that are used to differentiate reinfection from recrudescence in clinical trials; 25 laboratories have been participating during the past 5 years, and 7 are enrolled for the next testing round. WWARN has developed a scheme to improve malaria microscopy standards for research and is considering creation of EQA schemes for specific markers of antimalarial resistance.

The results of a quality assurance experiment for the performance of PCR assays in three laboratories and the comparability of the results were presented. The test panel for the experiment was prepared with cell suspensions from the Singapore Immunology Network, containing individually sorted P. falciparum-infected red blood cells from ring-stage synchronized culture. The suspension of infected red blood cells was diluted and added to packed cells obtained by depletion of plasma and buffy coat from whole blood to attain equivalent concentrations of 13 852 p/mL, 1385 p/mL, 346 p/mL, 139 p/mL, 69 p/mL, 35 p/mL, 14 p/mL and 7 p/mL. Negative controls containing no parasites and samples spiked with purified P. vivax DNA were included in the panel of 112 frozen samples sent to all three laboratories. The results from the three laboratories were in excellent agreement and displayed limits of detection in the range of 16–60 p/mL, well below the pre-defined pass criterion of 100 p/mL. This initial test will be complemented by a further round of tests in November 2015 with samples of concentrations ranging from 10 000 to 5 p/mL in a 96-well format.

It was noted that use of frozen whole blood without RNA stabilizers, as in this experiment, might preclude its use in RNA-detecting NAATs because of the lower stability of RNA; this could be obviated by sorting parasites directly into RNA-stabilizing reagents. Further, the cost of production of these materials is high and may not be appropriate for laboratories that will validate NAATs for routine or clinical diagnosis. Nevertheless, this approach has distinct advantages for ultrasensitive assays (lower limit of detection, ≤ 100 p/mL) used in the detection of submicroscopic infections in research or epidemiological studies or for testing or validating new assays. As individual infected red blood cells are deposited by fluorescence-activated cell sorting (FACS) into specific tubes or wells of multi-well plates, the samples produced with this method minimize Poisson sampling effects, which become significant at very
low parasite densities. FACS-based parasite separation offers the added benefit of separating parasite stages into early rings, trophozoites and schizonts; similar methods for enrichment of gametocytes are being validated. Finally, these samples can be produced in any matrix (whole blood, packed cells with or without RNA stabilizers, frozen, dried spots, etc.) to suit common sampling or assay methods, and the method could also be adapted for P. knowlesi, which can be cultured and could potentially be used as an analogue for P. vivax. Such quality assurance materials or test panels would be useful for laboratories that wish to certify the performance of ultrasensitive NAATs and for assessing or validating the analytical sensitivity of new assays.

3. ASSESSMENT OF NEEDS FOR EXTERNAL QUALITY ASSESSMENT

3.1 Survey of practices and needs

In order to evaluate current NAAT practices and the perceived need for an international NAAT EQA scheme, the WHO Global Malaria Programme, in collaboration with the University of Washington (USA), designed and conducted an online survey in 2014. Potential participating malaria laboratories were contacted through various communication networks, including WHO regional offices. Responses were obtained from 56 laboratories, most of which are located in Africa and Europe, but also including laboratories in Bangladesh, Brazil, Colombia, India and Japan and other countries in South-East Asia.

The responding laboratories reported that they used various NAATs, including nested PCR (23%), quantitative and qualitative real-time PCR (24% and 15%, respectively), qualitative PCR (15%), reverse-transcription PCR (14%), loop-mediated isothermal amplification (7%) and other NAATs (2%). Half of the respondents were not aware of the existence of a WHO international standard for P. falciparum DNA, while 32% (n=9) of those that were aware of it reported that they used it. When asked about the most appropriate sample format for their NAATs, most respondents reported whole blood (40%) and dried blood spots (DBS) (36%); RNA and lyophilized whole blood samples were also used (18% and 6%, respectively). When asked about what samples were considered acceptable, lyophilized whole blood was mentioned by 15% of respondents, probably because they can use such samples even though they usually do not lyophilize samples before processing them. The majority of respondents mentioned whole blood (36%) and DBS (32%) as acceptable samples. Laboratories were not asked whether “acceptable” meant that the sample type had been validated in their laboratory. The target genes reported included 18S rRNA (57%), resistance markers (24%) and gametocyte-expressed genes (11%).

Most of the responding laboratories conduct 100–1000 (38%) or 1000–10 000 (43%) tests per year, while a few reported performing fewer than 100 (10%) or more than 10 000 (9%). NAATs are typically used in clinical trials (33%), epidemiological studies of asymptomatic malaria (24%), resistance monitoring (20%), CHMI studies (12%) and clinical care (9%). Slightly less than half of the respondents reported that they would not agree to contribute financially to a NAAT EQA scheme (42%), while an approximately similar number would be ready to invest up to US$ 500 per shipment (43%) and a few up to US$ 1000 (9%) and US$ 2000 (6%). Involvement in a formal or informal EQA system was reported by 46% of respondents. Interestingly, the proportion of respondents who were ready to contribute financially to an EQA scheme was higher among those that had already participated in an EQA scheme.
(78%, n=18/23) than among those that had not (45%, n=13/29), suggesting that laboratories experienced in EQA are aware of the added value of such schemes.

Comments on the ideal characteristics of an EQA scheme included: an open, transparent, affordable, fair system; timely, clear information about sample shipment; rapid feedback of results; and periodic, routine publication of results for country-specific accreditation programmes. When asked about the ideal frequency of sample shipment and the number of samples per shipment, most opted for two to three shipments per year (68%) with 1–10 samples per shipment (52%), while some favoured a panel of 11–50 samples per shipment (36%).

3.2 Customer requirements

An open discussion was held about potential customer requirements for an NAAT EQA scheme. The findings in the survey described above that customers might not be ready to invest substantial financial resources into an NAAT EQA scheme will directly affect the type of material and scheme structure. The possibility of an evolving scheme was discussed. Initially, a simple, low-cost scheme could be developed, which could eventually respond to more specific needs, such as RNA- or gametocyte-containing samples. An evolving scheme would have the advantage of promoting customer feedback to ensure the success and effectiveness of the scheme. A dynamic scheme might better respond to changes in NAAT practices that might emerge as a result of testing the performance of various NAATs against a common reference panel.

It was generally agreed that, because of the diversity of methods in use, NAAT EQA samples should allow evaluation of the performance not only of qualitative assays but also quantitative ones. They should therefore include samples of varying parasitaemias with all human-infecting Plasmodium species, as well as negative samples. EQA samples should not, however, be considered as calibrating samples or allow calculation of limits of detection (LODs) more precisely than orders of magnitude. The group agreed that more precise calibration is the responsibility of individual laboratories. Sample parasite concentrations should be based on the sensitivity of common assays and include one order of magnitude below the current LODs in order to ensure that most of the positive samples provided would be informative.

Lyophilized whole blood samples were seen as the ideal format, to avoid costly refrigerated transport and frozen shipments, while at the same time closely resembling real samples – an essential requirement of EQA schemes – and ensuring their stability and safety. DBS should also be considered if they are the type used in the laboratory; furthermore, DBS prepared according specific procedures would be amenable to RNA extraction and are relatively cheap to prepare and distribute.

4. DEFINITION OF MATERIALS FOR EXTERNAL QUALITY ASSESSMENT

A central element of EQA schemes is the nature of the samples used to assess quality. EQA samples necessarily have a known, but undisclosed, content and must resemble typical samples as closely as possible. The deliberations of the participants concerning the characteristics of an ideal sample panel for NAAT EQA are summarized below and in Table 1.
Two main types of sample were discussed: whole parasites in blood (cultured or clinical) and synthetic DNA or RNA molecules. Although synthetic nucleic acids could be produced in a standardized manner relatively easily and present no biohazard, it was generally agreed that blood samples containing whole parasites best resemble real samples and should be the preferred material for NAAT EQA at the outset.

With regard to use of cultured or clinical material, the former allows more control over the system, whereas the latter must be processed quickly and a sufficient volume of infected patient blood must be available to stock and replenish the repository. Several conditioning options were considered: freezing (with and without the specific stabilizing buffers), lyophilizing and storage as DBS. The higher storage and shipping costs for frozen material led participants to strongly favour conditioning options that allow storage and shipping at ambient temperature, such as lyophilized samples and samples immobilized as DBS. It was noted that lyophilized whole blood sample are well suited for RNA-based NAATs, while DBS are acceptable. DBS have the advantages of being compact, affordable and with no major biohazard liability; however, a single DBS contains a limited volume of sample (~50 µL/spot), of which only a fraction is used during NAAT sample processing. The stability of DBS should be investigated; one participant found that DBS packed with desiccant are stable for at least 1 year at room temperature.

With regard to species representation in an evolving scheme, it was generally accepted that samples of *P. falciparum* and *P. vivax* should be included at a minimum and that the other human-infecting *Plasmodium* spp., *P. ovale*, *P. malariae*, and *P. knowlesi*, might be added to sample panels subsequently.

The source of parasites was also discussed. Use of culture-adapted standard laboratory *P. falciparum* strains was considered feasible; however, patient isolates would be closer to real samples and should be favoured. It was duly noted that there is no robust laboratory culture system for non-*P. falciparum* species, and patient isolates will have to be used.

The complexities added by the *Plasmodium* life-cycle were discussed. First, the DNA content of a single *Plasmodium* parasite differs widely according to its life-cycle stage; samples should ideally contain only ring-stage parasites with a single haploid genome. Secondly, red blood cells can be infected by multiple parasites, which affects quantitative reporting of the number of parasites per volume of blood and can shift the expected distribution of positive results when the sample is diluted to obtain low-parasitaemia control specimens. Samples containing gametocytes and prepared under RNA-preserving conditions were considered to be potentially useful but not essential.

In a discussion on the range of parasitaemia to be included in EQA samples, it was agreed that a single sample panel that could be used for NAATs with various LODs would be preferable to multiple panels specifically designed for given NAATs. The number of uninformative samples with parasitaemias below the LODs of the majority of malaria NAATs should be minimized. Therefore, it was agreed that samples should have parasite concentrations of 2 000 000 p/mL, 200 000 p/mL, 20 000 p/mL, 2000 p/mL, 200 p/mL and 50 p/mL. The highest concentration represents the parasitaemia seen in patient samples, while the lowest densities will indicate the performance of NAATs near the LODs achieved in most laboratories. Panels of 10 samples were considered appropriate, with six positive samples at the concentrations listed above and four negative samples. Addition of negative samples contaminated with other parasite species was discussed and considered not to be a priority at this stage but potentially useful subsequently. Quarterly testing was considered to be most appropriate for starting the EQA scheme.
5. Structure of External Quality Assessment Scheme

5.1 Candidate Suppliers

On the basis of their expertise in providing samples for malaria NAAT EQA schemes, UK NEQAS and WWARN were considered candidate sample suppliers.

UK NEQAS is legally part of Public Health England, under the supervision of a steering committee and an advisory panel. The Service already conducts several schemes, including faecal and blood parasitology and parasite and Toxoplasma serology. In some of these schemes, an EQA scheme has led to an overall improvement in laboratory performance. A malaria EQA scheme will respond to the requirements of the British Clinical Pathology Accreditation Standard. New schemes are developed during pre-pilot and pilot phases before becoming fully functional. The pre-pilot phase serves to define the type and source of materials and the evaluation criteria, while the pilot phase is extended to a larger set of participants to allow compliance with design criteria. Full schemes are introduced after approval by the steering committee and advisory panel. The development of new schemes is usually covered by UK NEQAS funds until they become self-sustainable with financial contributions from participating laboratories.

The main difference from the scheme being prepared by the WWARN is use of cultured parasites enriched by FACS-based methods, which allows precise control over the parasite densities, stages and strains used in test panels and the choice of matrix (whole blood, packed cells, DBS, with or without RNA-stabilizing reagents). An additional advantage of using cultured parasites with known polymorphisms is that they could be used to test the proficiency of other NAATs for genotyping. The species would be largely restricted to *P. falciparum*, although adapting the FACS-based parasite enrichment for cultured *P. knowlesi* is likely to be practicable, and this approach might eventually be applied to patient samples. This flexibility is seen as ideal for accommodating specific needs and the various uses of NAATs but would be comparatively costly, in particular due to shipping costs for frozen samples, for EQA scheme participants performing NAATs for routine or clinical diagnosis. It would nonetheless be useful for laboratories in developing new assays or validating the performance of their existing ultra-sensitive NAATs.

5.2 Advisory Group and Referee Laboratories

Setting up an advisory committee is one of the key steps in establishing an EQA scheme. This committee is responsible for designing, planning and implementing the scheme, and it should comprise the EQA scheme organizer, experts in molecular-based malaria diagnosis, representatives of the institution that supplies samples for testing panels, health authorities, participating laboratories and professional bodies. Once the EQA scheme is established, the advisory committee should continue to give direction for its effective continuation. It will set policies on the strategy of the scheme, the rules of participation, the tests to be included for assessment, the methods by which the definitive “correct answers” will be established, the principles of scoring and defining poor or unsatisfactory performance, action to be taken on unsatisfactory performance, the complaints procedure, promotion of the scheme and the role of the scheme in education and training. The advisory committee also provides professional, scientific and medical guidance on operational matters, including planning the aims and content of each exercise, agreeing on the content of reports, reflecting the views of participating laboratories, reviewing complaints and promoting the educational and training role of the scheme.
The proposed overall structure of a malaria NAAT EQA scheme would include oversight from WHO to ensure management of the advisory committee, advocacy, training and remediation. A single, central repository is most appropriate for sourcing and characterizing specimens in collaboration with partner laboratories, performing pre- and post-distribution checks, collating results, issuing reports and handling queries. Specimen distribution could be organized through regional hubs, and additional material that is relevant locally could be included or integrated into existing national schemes.

Referee laboratories are required to reach consensus on the correct results for each sample. They could be selected on a rotating basis with the following criteria:

- a high quality rating (preferably ISO 15189 or accredited by a recognized regulatory agency for medical laboratories);
- perform NAAT assays frequently;
- participate and achieve satisfactory results in EQA schemes for molecular diagnostic testing;
- perform representative assays for malaria (quantitative, qualitative);
- are in the same geographical region as primary participating centres (i.e. Africa, Asia and North America);
- have established working relations with in-country stakeholders;
- have staff for the additional workload; and
- have no significant problems in importing material.

5.3 Distribution of materials for external quality assessment

The distribution of EQA materials was discussed in relation to the procedures in place at the Australian National Reference Laboratory (NRL), which was established in 1985 as part of the Australian Government’s HIV/AIDS strategy to evaluate HIV tests and adjudicate on interpretation of their results. The NRL is a not-for-profit WHO collaborating centre for laboratory testing services, including comprehensive quality assurance schemes, evaluation of tests and test algorithms, training with sustainable outcomes, and consultation and advice on policy for laboratory testing.

As part of its quality assurance activities, NRL provides EQA schemes for various serological or NAA tests for infectious diseases using the Internet to collect and analyse data (www.oneworldaccuracy.com). The elements to be considered for a cost-effective approach to an EQA scheme include consultation with other EQA providers, use of in-country distributors, production of multiple panels at a time, adequate storage stability and use of Internet systems for data collection so that participants can enter their data into templates and forms to ensure consistent information.

For practical shipment of material, the following points were considered of particular importance:

- Samples must be shipped on a predetermined schedule with advance notice, and deliveries must be tracked.
- The shipment temperature should be recorded and/or tracked.
- Follow-up must be triggered if the shipment is not received at the scheduled time.
A plan must be drawn up to ensure enough reagents and supplies.

Shipments of samples must be inspected upon arrival to verify their content.

The EQAS provider should be notified if shipments are damaged or delayed, and replacement material should be provided.

Customs and import requirements must be adequately prepared.

Generally, shipments must comply with national and international regulations, including those of the International Air Transport Association for dangerous goods. The NRL uses the OASYS tool from Oneworld Accuracy to manage the procurement and shipment of specimens to scheme participants, taking advantage of the worldwide distribution of collaboration members.

### 5.4 Data management and reporting

The data management and reporting practices of the NRL were presented and discussed. The aim of data collection, the type of data, the type of analyses and the procedures for data collection and management must be established in a clear, transparent manner. Thus, the method of collecting data and a valid statistical analysis plan should be established before data collection. Data should be collected in a standard format in a complete, unambiguous, accurate, timely way. Those to be collected usually include:

- sample condition on receipt;
- the assays used, including details such as LOD and limit of quantification;
- the extraction, amplification and detection procedures;
- the instruments used and operator details; and
- raw and converted values with adequate units.

A web-based data collection system is more expensive to set-up than a paper-based system but facilitates the collection of data and minimizes the work of the EQA scheme provider. Data management is an essential aspect of an EQA scheme; it is complex and must be adequately implemented and documented to ensure the integrity of data and its long-term maintenance.

### 5.5 Performance requirements

NAAT performance requirements depend on the primary indication for performing NAAT, e.g. to support CHMI studies or clinical case management. In all cases, the amount of blood used to perform the assay is a critical variable; assays run from DBS samples may include as little as 1 µL of blood. Laboratories performing CHMI studies work at high proficiency levels and aim to harmonize procedures and share knowledge, on the basis of international standards and guidance documents.

The capacity to detect sub-microscopic parasitaemia is essential in laboratories in malaria-endemic countries conducting clinical or epidemiological studies for the control or elimination of malaria. It was proposed that NAATs should robustly detect parasitaemia ≥ 10 000 p/mL for clinical case management and 500 p/mL for epidemiological studies, but consensus was not reached on these values, although they are within the range of parasitaemias proposed for an optimal sample panel (Table 1). It might be necessary to define more precisely the expected LOD of malaria NAATs and the limit of tolerance beyond which an assay would not be considered of acceptable quality.
5.6 Remediation of unacceptable performance

A clear scoring system for assay parameters should be defined to assess e.g. species identification, positivity and quantification of parasitaemia, and to identify levels of performance. The system should be unbiased and quantitative; for instance, correct species identification could be granted two points, an equivocal result one point and a false-negative or false-positive result minus one point, to avoid universal equivocal reporting. It was generally agreed that a numerical scoring scheme should be defined, but this was not elaborated further.

The UK NEQAS scheme typically alerts laboratories that are performing in the lower 5th percentile by writing and includes them in an error report scheme in order to identify the causes of the poor performance. Laboratories that repeatedly perform poorly receive additional alerts, until a formal procedure is initiated, potentially leading to their de-licensing. Remediation to avoid such cases can be conducted in several ways, including feedback on error reports, expert advice from peers by post, telephone or in person during a laboratory visit; each option has a budget and human resource requirements. It was generally agreed that laboratory visits for remediation could not be conducted in a global malaria NAAT EQA scheme but that local expertise could be used. Even though the scheme should provide the tools and means to remediate poor performance, the laboratory is responsible for ensuring remediation. The sponsors of these laboratories might then allocate appropriate resources to investigate errors and improve the quality management system.

5.7 Participation fees

The fees that might be requested from the participants in a malaria NAAT EQA scheme should be included in the funds available to balance the costs for establishing and maintaining the scheme (Table 2). It was agreed that an NAAT EQA scheme should be self-sustainable beyond the launch phase, which might require one-time support from donors. The cost of establishing and maintaining a NAAT EQA scheme was not determined; it might be based on the results of the UK NEQAS pre-pilot exercise, which should become available in September 2015. It was generally agreed that participants would have to contribute financially to the scheme, possibly depending on their resources and location.

6. COMPLEMENTARY MATERIALS AND ACTIVITIES

6.1 International standards

Malaria biological standards and their potential use in a NAAT EQA scheme were discussed. Biological standards are usually used to calibrate assays against an external reference standard, with arbitrary units that are not related to a specific measurement method but directly related to the physical standard. Secondary standards, calibrated against primary standards, are used as day-to-day runcontrols. An international *P. falciparum* DNA standard is available through the National Institute for Biological Standards and Control, and a proposal will be submitted to the WHO Expert Committee on Biological Standardization in October 2015 to prepare a standard for *P. vivax*. International standards for *Plasmodium* RNA assays or WHO reference reagents may also be considered. It was generally agreed that the use and availability of calibrators is not relevant for an NAAT EQA scheme, the goal of which is to evaluate the performance of assays in use, rather than to provide a calibration service. It was also agreed that provision of calibrators and controls is beyond the scope of an EQA scheme; laboratories should prepare their own controls.
6.2 Resource mobilization

Potential sources of funding for establishment of a malaria NAAT EQA scheme were discussed, and the following institutions were cited:

- the Bill & Melinda Gates Foundation
- the National Institutes of Health, USA
- product development partnerships (largely funded by the Bill & Melinda Gates Foundation):
  - Medicines for Malaria Venture
  - Malaria Vaccine Initiative
- the Global Fund to Fight AIDS, Tuberculosis and Malaria
- UNITAID
- regional organizations:
  - the Asia Pacific Malaria Elimination Network
  - the Gulf Cooperation Council
  - the Amazon Malaria Initiative

The rationale for sponsoring a malaria NAAT EQA scheme is to support elimination and evidence-based decision-making on elimination. NAATs of higher quality will directly improve case detection, enable better targeting of interventions, strengthen the capacity of clinical trial sites and facilitate the development of drugs, vaccines and diagnostics for malaria. The representative of the Bill & Melinda Gates Foundation reported that the Foundation is supporting development of a new molecular biomarker that could replace light microscopy and commented that an EQA scheme might be relevant to that initiative.

6.3 Future activities

The immediate next steps are to consolidate the recommendations of this consultation and prepare a resource mobilization plan with the associated responsibilities, as outlined in Table 3. The goal is to develop a sustainable WHO NAAT EQA scheme in partnership with appropriate operational and supporting institutions to meet the needs of the malaria community and contribute to malaria elimination by ensuring the quality of these highly sensitive assays.
### TABLE 1.
**Optimal sample characteristics for a malaria NAAT EQA scheme**

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>RECOMMENDATION</th>
<th>POTENTIAL ADDITION</th>
<th>COMMENT</th>
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<tbody>
<tr>
<td>Type</td>
<td>Whole parasites in blood</td>
<td></td>
<td>Not suitable for RNA</td>
</tr>
<tr>
<td>Conditioning</td>
<td>Lyophilization</td>
<td>Dried blood spot</td>
<td>DBS have only limited volume DNA is usually extracted from only part of a DBS DBS allow for RNA testing</td>
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<td>Species</td>
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<td><em>P. falciparum</em></td>
<td><em>P. malariae</em></td>
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<td><em>P. vivax</em></td>
<td><em>P. ovale</em></td>
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<td></td>
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<td><em>P. knowlesi</em></td>
<td></td>
</tr>
<tr>
<td>Source</td>
<td>Patient isolates</td>
<td>Containing gametocytes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prepared under RNA-preserving conditions</td>
<td></td>
</tr>
<tr>
<td>Parasitaemia coverage and controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 000 000 p/mL</td>
<td>Negative controls</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 000 p/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 000 p/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2000 p/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 p/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 p/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uncontaminated negative controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples per panel</td>
<td>10 samples (6 positive and 4 negative)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of testing</td>
<td>Ideally quarterly, minimum bi-annually</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 2.
**Potential financial sources and charges for a malaria NAAT EQA scheme**

<table>
<thead>
<tr>
<th>SOURCE OF FUNDS</th>
<th>COSTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allocation of a budget by government authorities</td>
<td>Infrastructure</td>
</tr>
<tr>
<td>Pairing with other EQA organizers</td>
<td>Information technology system</td>
</tr>
<tr>
<td>Fees from participants</td>
<td>Personnel</td>
</tr>
<tr>
<td>Short-term support from development partners</td>
<td>Reference material production, testing and storage</td>
</tr>
<tr>
<td></td>
<td>Material shipping and handling</td>
</tr>
<tr>
<td></td>
<td>Advisory committee meeting</td>
</tr>
<tr>
<td></td>
<td>Training and corrective action</td>
</tr>
</tbody>
</table>
### TABLE 3. Next activities and responsibilities

<table>
<thead>
<tr>
<th>ACTIVITY</th>
<th>RESPONSIBLE PARTIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirm target use and target use settings for EQA scheme:</td>
<td>All</td>
</tr>
<tr>
<td>• First: accelerated elimination efforts</td>
<td></td>
</tr>
<tr>
<td>• Second: product development and evaluation</td>
<td></td>
</tr>
<tr>
<td>Additional work on specimen characterization generally:</td>
<td>UK NEQAS, University of Washington (a), WWARN (b)</td>
</tr>
<tr>
<td>a. RNA preservation in lyophilized samples</td>
<td></td>
</tr>
<tr>
<td>b. panel of ultra-low parasitaemia samples</td>
<td></td>
</tr>
<tr>
<td>c. stability of lyophilized samples</td>
<td></td>
</tr>
<tr>
<td>d. availability of whole blood (instead of peripheral red blood cells)</td>
<td></td>
</tr>
<tr>
<td>e. addition of DBS as a sample type</td>
<td></td>
</tr>
<tr>
<td>Costs of the elements of the proposed EQA scheme</td>
<td>WHO Global Malaria Programme with help from (as needed):</td>
</tr>
<tr>
<td>• panels</td>
<td>• UK NEQAS</td>
</tr>
<tr>
<td>• distribution</td>
<td>• WWARN</td>
</tr>
<tr>
<td>• data analysis and management</td>
<td>• NRL</td>
</tr>
<tr>
<td>Technical advice and support (remediation and other purposes)</td>
<td></td>
</tr>
<tr>
<td>Refine performance expectations</td>
<td>WHO Global Malaria Programme</td>
</tr>
<tr>
<td>Support complementary activities</td>
<td>National Institute for Biological Standards and Control, University of Washington, WWARN</td>
</tr>
<tr>
<td>Identify referee laboratories</td>
<td>All</td>
</tr>
<tr>
<td>Determine interest of possible funders</td>
<td>All</td>
</tr>
<tr>
<td>Refine message and write proposals</td>
<td>WHO Global Malaria Programme</td>
</tr>
</tbody>
</table>
ENDNOTES


7. UK NEQAS has access to 100 malaria patients per year at the Hospital for Tropical Diseases in London and 900 samples per year from the mycology reference laboratory (with more fresh samples then from the Hospital for Tropical Diseases).