

WHO external quality assurance scheme for malaria nucleic acid amplification testing

Operational manual

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Abbreviations

DBS	Dried blood spot
EQA	External quality assurance
FIND	Foundation for Innovative New Diagnostics
GMP	Global Malaria Programme
HTD	Hospital for Tropical Diseases in London
ISO	International Standardization Organization
LOA	Letter of agreement
LOD	Limit of detection
KEMRI	Kenya Medical Research Institute
NAA	Nucleic acid amplification
NAAT	Nucleic acid amplification testing
NHSBT	National Health Service Blood and Transplant in the UK
PCR	Polymerase chain reaction
PHE	Public Health England
PT	Proficiency testing
QC	Quality control
TOR	Terms of reference
UK NEQAS	United Kingdom National External Quality Assessment Service
WHO	World Health Organization

1 Background

Nucleic-acid amplification (NAA)-based assays are increasingly being used in the context of malaria epidemiological surveys and research, particularly as diagnostic endpoints in clinical trials of vaccine and drug candidates. New methods are also being developed for various applications, including tools to be used in the context of malaria elimination efforts. Because NAA-based tests (NAATs) have superior sensitivity compared to microscopy and rapid diagnostic tests (RDTs), they are particularly useful for detecting low-density (<100 parasites/μL) infections, which are often asymptomatic but contribute to malaria transmission. A plethora of malaria NAATs have been reported in the literature, but results show variation in performance from one study to another.

The role of malaria diagnostics in low transmission settings was reviewed at a WHO Evidence Review Group meeting in December 2013. One of the conclusions drawn was that an international external quality assurance (EQA) system needed to be established in order to ensure that data obtained from these assays were reliable and comparable [1]. A follow-up expert group meeting on establishing a WHO EQA scheme for malaria NAAT was held in June 2015 in London, UK. Experts in malaria molecular testing and EQA reached a consensus on the desired characteristics of a WHO international EQA scheme for malaria NAAT [2]. The scheme was launched in 2017.

2 Purpose

The purpose of the WHO malaria NAAT EQA scheme is to offer an independent and periodic means for clinical, reference and research laboratories to verify the quality of their NAA-based malaria diagnostic methods and to monitor performance over time. This is achieved through the provision of well-characterized and quality-controlled panels consisting of a blinded mix of *Plasmodium*-positive and -negative samples. Participants are then issued an EQA report upon submission of their analysis results. Participation in the scheme is voluntary, and results are confidential.

The scheme will enable laboratories to obtain an independent assessment of their NAA-based diagnostic methods and thereby determine if they meet the minimum level of quality. If any laboratory does not meet this requirement, some level of remote technical support will be offered, and the laboratory can work on identifying and addressing any source of errors in order to improve the quality and reliability of their methods.

The system is not designed to deliver “good or bad marks” to participants or to license laboratories. On the contrary, the scheme is educational and aims to reinforce mutual confidence within a network of laboratories. Ultimately, the scheme aims to strengthen and improve standards of performance of malaria NAAT in laboratories supporting a range of malaria-related research. Improved performance in NAAT will ensure that WHO policy making is based on the highest quality evidence, and interventions and resources are most appropriately targeted.

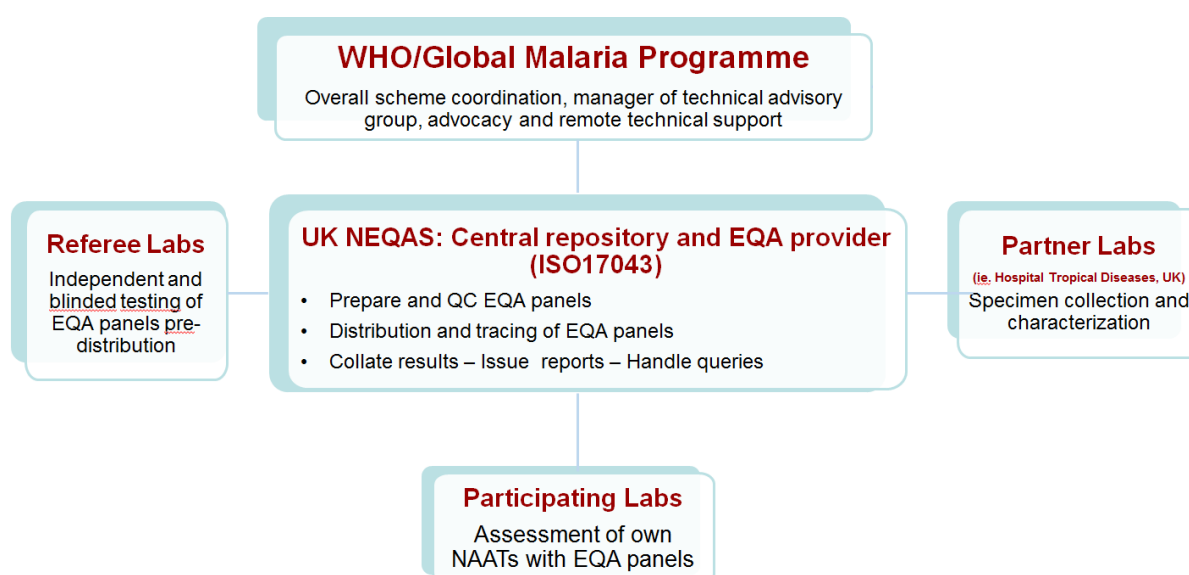
3 Objectives of this document

- i. To outline the administration, functions and processes¹ of the WHO malaria NAAT EQA scheme;
- ii. To clarify the responsibilities of the repository holder regarding the receipt, registration, characterization, preparation, storage, management and distribution of EQA panels;
- iii. To clarify the responsibilities of the NAAT EQA Advisory Group, the referee laboratories and the participating laboratories;
- iv. To define data management practices.

4 Management and structure of the NAAT EQA scheme

The WHO malaria NAAT EQA scheme is coordinated by the WHO Global Malaria Programme (WHO GMP), in collaboration with Public Health England (PHE) through the United Kingdom National External Quality Assessment Service (UK NEQAS). An overview of the structure is shown in Figure 1 below.

Figure 1: Overall structure of WHO malaria NAAT EQA scheme



WHO/GMP is responsible for the overall coordination of the scheme, organizes regular meetings of the Advisory Group, and promotes the scheme. UK NEQAS currently holds the central repository of EQA materials and, based on agreed terms, manages the scheme operations. This includes storage and shipping of EQA panels; the issuance of EQA reports to the participating laboratories; and the handling of any logistical queries via organiser@uknegasmicro.org.uk. Preparation and characterization of materials is conducted at partner laboratories.

Referee laboratories conduct independent testing of EQA panels prior to general distribution. The selection of referee laboratories is based on defined criteria, as described under the terms of reference

¹ Field collection of specimens, shipment to repository, storage, requests for specimens and material release (to end-users)

(TOR) for referee laboratories (Appendix 1), and their participation is voluntary. The final characteristics of each panel are based on a high-level consensus of results coming from the referee laboratories.

Participating laboratories are enrolled following response to a call for interest and after having signed a letter of agreement (LOA) with WHO. Registered laboratories receive EQA panels from UK NEQAS on a 6-monthly basis and enter their results via a web-based database. Subsequently, UK NEQAS issues reports through the web portal. In the case of substandard results (described in section 11 “Troubleshooting and remedial action”), it is recommended that participating laboratories complete an incident review form and conduct a root cause analysis. Remote technical support and remediation can also be provided through WHO and/or referee laboratories but it is limited.

An Advisory Group is convened at least yearly to review the scheme procedures, provide technical expertise for potential modifications to procedures and principles, review overall results of the scheme, including complaints, and discuss actions required to address major issues, if any. The TOR of the Advisory Group are outlined in Appendix 2.

5 Preparation, characterization and storage of EQA samples

The EQA materials are prepared for WHO in the UK NEQAS premises based at the Halo building, London. *Plasmodium*-positive samples are prepared from cultured parasites (*P. falciparum* and *P. knowlesi*) or from clinical blood samples, while *Plasmodium*-negative blood samples are prepared from healthy blood donors. Currently, two types of EQA samples are made available: lyophilized blood samples and dried blood spot (DBS) samples.

- Cultured parasites

Cultured strains of *P. falciparum* and *P. knowlesi* are available through the London School of Hygiene and Tropical Medicine. They are cultured according to standard procedures, and the parasitaemias of culture batches are determined by expert microscopists. *P. falciparum* parasites are synchronized to ring-stage, while *P. knowlesi* parasites are left asynchronous. Parasites are then diluted to the target parasite densities using blood samples from National Health Service Blood and Transplant (NHSBT) that are confirmed to be parasite-negative by microscopy and nested PCR.

- Parasites from clinical samples

Blood samples are obtained from patients infected with *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*, as available. (Specimens collected for EQA purposes are exempt from signed informed consent according to the UK Human Tissue Act [3].) These clinical samples are characterized by expert microscopists in order to determine parasite density and the species present. PCR is used later to confirm the presence of single species. Blood samples are then diluted to the target parasite densities using blood samples from NHSBT that are confirmed to be parasite-negative by microscopy and nested PCR.

- Negative control samples

These are prepared from blood samples from NHSBT that are confirmed to be parasite-negative by microscopy and nested PCR. The blood is obtained as bags of citrate-anticoagulated blood.

- Dilution of parasite samples

Ten-fold serial dilutions are prepared using 20 mL syringes (for cultured parasites) or calibrated micropipettes (for clinical samples) and mixed by thoroughly repeated up-and-down pipetting followed by mixing using IKA Loopster Digital mixer for at least 10 minutes at 35 RPM. Work is done at room temperature in a Class II safety cabinet. Diluted samples are then frozen at -80°C for subsequent aliquoting.

At UK NEQAS Parasitology premises, parasites are diluted in citrate-anticoagulated whole blood obtained from NHSBT. A bag of whole blood (up to 500 mL volume) is decanted into a 500 mL square plastic wide-mouthed bottle. Aliquots of 90 mL of whole blood are poured from this bottle into 100 mL square plastic wide-mouthed bottles. The parasite sample is then removed from the safety containers and the centrifuge tube is inverted several times to mix the sample thoroughly. Using a 20 mL syringe, 10 mL of the parasite sample is removed from the tube and placed into the first of the 90 mL blood aliquots. Then, using the syringe, the sample is mixed in with the whole blood by filling the syringe and releasing the contents several times back into the same 100 mL bottle (for at least 5 minutes to mix thoroughly). Using the same syringe, 10 mL of infected blood is removed from the bottle and placed into another bottle with a 90 mL aliquot of whole blood. This is similarly mixed using the syringe. These 1:10 dilutions continue until all desired parasitaemias are achieved. For each dilution step, further mixing is carried out using IKA Loopster Digital mixer for at least 10 minutes at 35 RPM. A bottle containing 100 mL negative blood for making dilutions on the day is also stored. Each bottle is labelled with the (1) parasite species, (2) date of sample preparation and (3) number of parasites per mL. After aliquoting for DBS preparation, the dilutions are stored at -80°C for long-term storage.

- Preparation of DBS samples

At the UK NEQAS bench, DBSs are prepared on Protein Saver 903 cards following a protocol provided by Dr Sean Murphy of the University of Washington Medical Center. Typically, 75 DBS cards are prepared per sampling. Each card is labelled with "DBS xx" and the date of the sampling, where DBS stands for "dried blood sample" and the xx refers to the number of the sample (i.e. the first DBS prepared is DBS01, the next DBS02, and so on).

While wearing gloves, all 75 cards are opened to reveal the filter paper in the open position (no flap over the blood spots). Using a sterile tip, 50- μ L aliquots are removed from the appropriate parasite dilution or parasite-free control and placed into the centre of a circle on the card where the sample is to be deposited. The sample is absorbed into the filter paper, typically filling the entire circle. The samples include two negative controls (no parasites) and two parasite dilutions across the three remaining circles. The precise positioning of the spots, dilutions aliquoted, and parasite species used are noted in the inventory. The spots are then laid out inside a Class II cabinet and dried for 3–4 hours. Thereafter, the cards are flipped into the closed position (covering the filter paper with the cardboard sleeve). These are placed into a gas impermeable bag with desiccant and placed in a freezer at -80°C for long-term storage.

- Preparation of lyophilized panel samples

The diluted blood samples are thawed and mixed using IKA Loopster Digital mixer for at least 10 minutes at 35 RPM, then aliquoted in 0.5 mL volumes using a repeater pipette in glass vials for subsequent freeze-drying in a freeze-drying machine. The samples are all quality-checked prior to distribution. Once freeze-dried, the specimens are stored at -20°C or -80°C.

- Quality control

All samples (both freeze-dried and DBS) are quality-controlled by referee laboratories prior to distribution.

6 Testing of panels by referee laboratories and final status decision

Each batch of EQA panels is tested at the referee laboratories that are selected based on the pre-defined criteria outlined in Appendix 1. (Referee laboratories are also participating laboratories in this scheme.) The sample identities are hidden prior to shipment. One vial of each specimen and all the DBS cards to be used in the distribution are sent to the following referee laboratories:

- University of Washington Medical Center, Seattle, USA (Dr Sean Murphy)
- Institut Pasteur, Paris, France (Dr Didier Menard)
- Peter Doherty Institute of Infection and Immunity, Australia (Prof. James McCarthy)
- Kenya Medical Research Institute (KEMRI), Kilifi, Kenya Isabella Lynette Ochola, Melissa Kapulu)
- London School of Hygiene and Tropical Medicine (LSHTM), (Dr. Khalid Beshir/ Dr. Debbie Nolder)
- Health Services Laboratories LLP Analytics (Dr. Spencer Polley)
- Institute Pasteur, Cambodia (Nimol Khim, Jean Popovici)

Each referee laboratory uses its routine NAA-based malaria diagnostic methods to test each sample qualitatively (positive, negative, infecting species), including all necessary positive and negative internal controls to validate the testing experiments. Results are provided to WHO and the repository manager. The results from all referee laboratories and the QC results obtained after sample preparation at UK NEQAS are compared. Consensus for status as negative or positive and infecting species is reached when a clear majority of referee laboratories obtain identical results.

7 Composition of proficiency testing (PT) round panels

At each round, a panel of five lyophilized and five DBS samples is provided to participating laboratories. Each panel of 10 samples contains approximately six parasite-positive samples with a range of parasite densities from 50 up to 2 000 000 parasites per millilitre of blood (p/mL). The low end of this range will test performance close to the limit of detection (LOD) of most NAA-based diagnostic methods, while the higher end corresponds to parasite densities seen in naturally infected patients. Parasite-positive samples consist of both *P. falciparum* and non-*P. falciparum* samples as available.

8 Enrolment and registration of participating laboratories

Based on a survey issued to malaria research scientists and reference laboratories in 2014–2015, WHO compiled an initial list of potentially interested research, reference and clinical laboratories. All participating laboratories were asked to sign an LOA with WHO and to complete a laboratory profile template. Examples of these templates are shown in Appendices 4 and 5.

Requests to participate in or resign from the scheme should be sent to MalNAATEQA@who.int. New entries into the scheme are considered on a first come first served basis, dependent upon the capacity of the scheme. However, if demand significantly exceeds supply, prioritization will be defined by the Advisory Group based on the laboratories' primary testing indication and geography. Once laboratories return the completed address template and signed LOA, they are considered to be registered in the scheme for an unlimited period of time—until the laboratory wishes to stop participation, the

laboratory fails to submit results on 3 occasions or the scheme exceptionally needs to cancel participation because of capacity reasons.

9 Organization and process of PT rounds

About 4 to 8 weeks ahead of each PT round, new sets of panels are assembled and quality-controlled as described above. Upon passing the QC, samples are packaged in sets of five lyophilized and five DBS samples, ready to be shipped. All participating laboratories need to confirm their exact shipment details and ensure that they have shared the necessary permits for importing the EQA materials, which are classified as hazard group 3 (not screened for blood-borne viruses such as HIV or hepatitis B).

Approximately 1 week prior to the shipment, laboratories will be notified if shipment is going to be to airport only. Samples are shipped along with a set of instructions for use and handling, including storage precautions, a protocol for reconstituting freeze-dried specimens, and instructions for reporting results. Each laboratory is also provided with confidential login details to connect to a secure web-based portal where they can enter their results and access their final reports.

Laboratories are requested to immediately report to UK NEQAS (organiser@ukneqasmicro.org.uk) any issues with receiving the samples, such as delays due to customs or importation procedures. Upon receipt of the samples, laboratories typically have 5 to 7 weeks to enter their test results, depending on the efficiency of shipment. A precise deadline for submitting results (maximum 8 weeks after distribution) is communicated to participating laboratories via email and by letter accompanying the PT panel. Results need to be entered along with some information about the amplification target (DNA or RNA) and methods used for nucleic acid extraction and amplification. Laboratories are encouraged to use their routine DNA extraction and amplification methods for testing the samples.

Laboratory results appear on the [UK NEQAS website](#) under WHO Malaria Molecular and the distribution identification number within one week following the deadline for submission of results. A report containing the laboratory's results for each specimen and assessment scores, along with any recommendations for further action, is uploaded to the secure portal by UK NEQAS approximately 3 weeks later. Laboratories that fail to test the samples or enter the results within the allocated timeframe will not receive a report; they may be contacted to identify the source of the problem and make a decision about the laboratory's participation in subsequent PT rounds.

10 Scoring system

Results obtained for each individual sample are scored according to the scoring scheme presented in Appendix 3. The scores are adapted to the capacity of the laboratory's method to determine only *Plasmodium* infections, only *P. falciparum* infections, or the full range of answers, i.e., to identify *Plasmodium* infections and determine each of the human infecting species. The rationale of this scoring scheme is to acknowledge 100% correct results with a score of '2', taking into account the level of "correct" results that can be obtained by laboratories using methods with limited capacities. For example, for a method detecting *P. falciparum* infections only, the non-detection of a *P. vivax* or *P. knowlesi* sample is not rated as a false-negative result. False-positive or false-negative results are penalized with a score of '-1' in all cases. Incorrect species identification but correct recognition of a *Plasmodium* infection is rated with a score of '0'. Indeterminate results are likewise assigned a score of '0' to acknowledge the fact that such results are commonly obtained when nearing the LOD of NAA-based methods.

The final answers for all samples will be used to calculate the final summary score, which reflects the overall performance of each laboratory. Laboratories scoring within 1.96 standard deviation of the mean are considered to meet the minimum level of quality required for the use of NAA-based malaria diagnostic methods. Laboratories with lower scores are strongly urged to work on improving their performance.

11 Troubleshooting and remedial action

In the case of any substandard results, laboratories are recommended to complete an incident review form, which is made available on the UK NEQAS website. To help identify the source of the quality issues, laboratories can request a repeat panel to be shipped for re-testing and further investigation. Laboratories are also put in contact with an expert who can provide remote technical support via email or phone throughout the process of investigating and addressing the problem. Other options include putting the laboratory in contact with other laboratories from the network that might have faced and solved similar problems, and/or are using the same types of methodologies. It is the laboratory's right to decide if its EQA results can be shared with such third parties as part of the investigational process, and the laboratory can also freely decide whether to share other types of information, such as details of the instruments or reagents used for the diagnosis, and/or results from any repeat or troubleshooting experiments. Ultimately, it is the laboratory's responsibility to investigate and address the problem, with the aim of improving performance and achieving the required level of quality. The sponsors of the laboratory should consider allocating appropriate resources to investigate quality issues and to improve the quality management system of the laboratory, as required.

12 Data management and analysis

The data related to the status of each specimen and testing results of each laboratory are held in the dedicated web-based database, housed and managed by UK NEQAS. Only relevant staff from UK NEQAS and WHO have access to these data, and laboratories' EQA reports cannot be communicated to any third party without the written agreement of the laboratory's main contact person. Laboratories are free to communicate their testing results and/or EQA report to any third party as they wish.

UK NEQAS and WHO conduct analyses to monitor trends over time, both for individual laboratories and for the entire set of participants, in order to help identify and bring to attention any drifts in quality. Results can also be used to compare the performance of different nucleic acid extraction and/or amplification methods. In any case, UK NEQAS and WHO commit to communicating results in presentations, reports or publications in the peer review literature, only in an aggregated and anonymized manner in order to maintain confidentiality.

13 Communication and promotion of the EQA scheme

WHO is in charge of all the main communication to participating laboratories, such as sending calls for interest in participation, informing participants about data entry deadlines or availability of reports, and providing updates on the operations of the scheme. Communication of test results and EQA reports is only handled via the online secure web portal, unless laboratories wish to share their results and/or reports via other means to another party. WHO, UK NEQAS and the Advisory Group will exchange all necessary information to ensure the smooth functioning of the scheme, including the organization of

each PT round, remedial actions, and organization of regular meetings of the Advisory Group. Communications with partner and referee laboratories will be jointly conducted by WHO and UK NEQAS in handling the characterization and testing of reference samples. Information about the functioning of the scheme, including anonymized test results and/or reports, can be shared with the Advisory Group during the annual or ad-hoc meetings, if and as required for the Group to fulfil its role, as described in its TOR (Appendix 2).

A general description of the scheme's purpose, processes and operations is also provided publicly on the WHO and UK NEQAS websites, along with an aggregated version of the results to demonstrate the impact and benefit of the scheme. Other promotional communications will include presentations at international conferences or meetings and peer-reviewed publications in scientific journals.

14 Fees for participation in the scheme

Laboratories will be asked to pay a fee to participate in the scheme. This is to cover the costs of running the scheme including sample preparation, transportation of sample panels to labs and technical support. All fees charged are going directly to cover costs incurred from the running of the scheme. The fee to be paid by participating laboratories is on a tiered system, based on the country of the laboratory and type of laboratory. Countries have been classified as high, middle and low income, according to the World Bank classification. Laboratories in high income countries will be charged the highest fees while those in low income countries will be charged the lowest fees. Furthermore laboratories have been classified as private, research – mainly funded by research grants, or public/reference laboratory – mainly funded by public funds. Thus, two factors, country and lab type, have been put together to create a system of 4 tiers, as shown in appendix 6.

15 Additional *pfhrp2* and *pfhrp3* analysis

Pfhrp2 and *pfhrp3* gene deletions in *P.falciparum* parasites have been detected in many malaria endemic countries. These gene deletions allow the parasites to evade detection by HRP2-detecting RDTs and pose significant challenges for malaria case-management. Many laboratories are now conducting molecular analysis to determine if parasites have deletions of *pfhrp2/3* genes.

To enable laboratories which conduct this analysis to verify the quality of their *pfhrp2/3* gene deletion analysis, each panel may include *P. falciparum* samples with *pfhrp2* or *pfhrp3* gene deletions or both. Laboratories which conduct *pfhrp2/3* gene deletion analysis are welcome to conduct further molecular analysis on any samples they identify as *P.falciparum*, to determine whether the parasites in these samples have an intact or deleted *pfhrp2/3* genes.

16 Future evolution of the scheme

An area for expansion of the scheme is in the types of EQA panels provided to participants. For example, it is considered useful to include more diverse types of negative control samples, such as samples containing parasites other than *Plasmodium*. The inclusion of parasites a range of drug-resistance related mutations could be beneficial for laboratories conducting drug-resistance surveillance.

The implementation of these and other changes will be subject to discussions and decisions during Advisory Group meetings and the availability of funds. The Advisory Group plays a central role in this regard; it is best placed to suggest future orientations, as it includes representatives from all relevant stakeholders in the scheme, who can appreciate not only the scientific interest and need but also practical feasibility of each change.

16 References

1. WHO Evidence Review Group on malaria diagnosis in low transmission settings, meeting report, 16-18 December 2013. Geneva: World Health Organization; 2014.
2. A WHO external quality assurance scheme for malaria nucleic acid amplification testing, meeting report, 8–9 June 2015, London, UK. Geneva: World Health Organization; 2015.
3. Human Tissues Act 2004
(http://www.legislation.gov.uk/ukpga/2004/30/pdfs/ukpga_20040030_en.pdf).

Appendix 1 – Terms of reference for referee laboratories

Background

In 2016, WHO established an international external quality assurance (EQA) scheme for NAA-based malaria diagnostic methods, with a central repository and scheme manager responsible for the preparation, characterization and QC of the EQA materials. Once the reference panels are prepared, their status needs to be confirmed by independent testing conducted by referee laboratories of high quality. These terms of reference describe the criteria for selecting referee laboratories for this scheme, as well as the laboratories' roles and responsibilities.

Criteria for selecting referee laboratories

- Laboratory of a high quality standard (complying with GCLP guidelines and preferably having international standard ISO 15189 for medical laboratories)
- Participation in and achievement of satisfactory results in EQA schemes for molecular diagnostic testing
- Performance of representative molecular assays for malaria
- Geographical overlap with laboratories participating in the scheme, i.e. North and South America, Africa, Asia, Western Pacific
- Demonstrated working relationship with endemic country stakeholders
- Appropriate staffing to accommodate additional workload
- No significant importation issues, e.g. no restrictions on receipt of samples not screened for blood-borne viruses such as HIV, hepatitis B or C

Roles and responsibilities

The proficiency panel prepared locally by the EQA repository and scheme manager should be verified by a referee laboratory to ensure that the end product is consistent with the planned product, and to ensure uniformity and adherence to standard procedures of preparation. Verification should be done for all panel samples that are planned to enter the panel distribution rounds.

Once enrolled, referee laboratories should engage in a contractual agreement with the EQA repository and scheme manager, and/or sign adequate Material Transfer Agreements, as required by the EQA repository and scheme manager and/or the referee laboratory. Before shipment of any proficiency samples, the referee laboratory should also ensure that any required import permits or other requirements are fulfilled for smooth transport and receipt of samples.

Once samples are received, the referee laboratory should test the samples using its routine testing procedures. The laboratory should also ensure that the quality of this testing procedure is assured by adequate internal and external quality control (IQC and EQC) measures, such as the use of negative and positive control samples in each experiment, and adherence to a regular EQA programme with a recognized institution.

Results should be communicated to the EQA repository and scheme manager within 1 week of panel receipt. In the case of conflicting results, the EQA repository and scheme manager may ask the referee laboratory to run confirmatory experiments.

Referee laboratories will be acknowledged in all relevant communication by WHO.

Appendix 2 – Terms of reference for the Advisory Group

Background

Following the recommendation of the 2013 WHO Evidence Review Group on malaria diagnostics in low transmission settings, an international external quality assurance (EQA) scheme for NAA-based malaria diagnostic methods was established in 2016 [1]. The overall structure of this EQA scheme includes an Advisory Group that is required for regular review of operations and participant results. The Advisory Group also guides any modifications to all aspects of the scheme based on a consensus of experts in the field and relevant stakeholders. These terms of reference describe the composition of the Advisory Group, as well as its roles and responsibilities.

Composition of the Advisory Group

The Advisory Group includes representatives of each of the parties that contribute to the functioning of the EQA scheme, e.g. the coordinator, the institution managing the scheme operations, and referee laboratories, in order to ensure that any decisions or recommendations of the group are agreed on by all parties. Furthermore, the participation of experts in NAA-based malaria diagnostics and/or EQA schemes for molecular tests for other diseases, as well as laboratories using NAA-based malaria diagnostics is required. The participation of these parties will ensure that discussions and decisions take into account the most up-to-date knowledge in these fields and that the scheme is responding well to the needs of laboratories performing molecular-based testing.

The membership of the Advisory Group is comprised of (recommended numbers of representatives are indicated in parentheses) [2]:

- EQA scheme coordinator, i.e. WHO GMP (1)
- EQA scheme manager, i.e. UK NEQAS, operated by Public Health England (PHE) (2)
- Representatives of:
 - Institution supplying bulk EQA material for panels: HTD (1) as well as UK NEQAS Parasitology (1-2)
 - Referee laboratories (2)
 - Public health authorities from malaria-endemic countries (1)
 - Participating laboratories (2)
 - Professional bodies (1–2)
- Selected experts in malaria molecular-based diagnostics and/or EQA schemes for malaria or non-malaria molecular testing (2)

Roles and responsibilities

Members of the Advisory Group will provide technical advice on all aspects of establishing, maintaining and revising the EQA scheme:

- For the set-up, contribute to the design, planning and implementation of the scheme:
 - Strategy and direction of the scheme

- Rules of participation
 - Tests to be included for assessment
 - Characteristics of the EQA panels
 - Methods for establishing the status of each sample
 - Selection of referee laboratories
 - Principles of scoring and defining poor or unsatisfactory performance
 - Actions to be taken in the case of unsatisfactory performance
 - Complaints procedure
 - Promotion of the scheme
 - Role of the scheme in education and training
- Once the EQA scheme is established, Advisory Group members will provide ongoing technical advice concerning:
- the aims and content of each exercise
 - the content of reports
 - complaints
 - proposed changes in procedures and/or other modifications to the scheme

Advisory Group members will also be expected to:

- Assist in analysing root cause(s) of poor performance through document review and discussions via email or telephone
- Promote the scheme and passively gather and share feedback from scheme participants

Functioning of the Group

The EQA scheme coordinator will call for at least one yearly meeting of the Advisory Group (maximum two) for a programme review. Meetings may be face-to-face or via teleconference, depending on Group member availability and funding. Ad-hoc meetings of the full or partial Advisory Group can also be organized to address specific needs, e.g. minor complaints or remedial actions.

References

1. WHO Evidence Review Group on malaria diagnosis in low transmission settings, meeting report, 16–18 December 2013, Geneva, Switzerland. Geneva: World Health Organization; 2014.
2. A WHO external quality assurance scheme for malaria nucleic acid amplification testing, meeting report, 8–9 June 2015, London, UK. Geneva: World Health Organization; 2015.

Appendix 3 – Scoring of results

1) For methods detecting *Plasmodium* and determining the species:

Positive specimens:

Code	Description	Report	Score
1	Genus and species correctly identified (e.g. <i>Plasmodium falciparum</i> nucleic acid present)	<i>P. falciparum</i> nucleic acid present	2
2	Correct genus but wrong species (incorrect <i>Plasmodium</i> species)	Incorrect <i>Plasmodium</i> species	0
3	Only <i>Plasmodium</i> genus identified (e.g. <i>Plasmodium</i> spp. detected)	<i>Plasmodium</i> spp. detected	1
4	Indeterminate result	Indeterminate result	0
5	No <i>Plasmodium</i> nucleic acid detected	<i>Plasmodium</i> spp. not detected	-1
6	<i>Plasmodium falciparum</i> nucleic acid not detected (relevant for labs doing <i>Pf</i> identification only)	<i>P. falciparum</i> nucleic acid not detected	-1

Negative specimens:

Code	Description	Report	Score
7	<i>Plasmodium</i> nucleic acid present	<i>Plasmodium</i> nucleic acid present	-1
8	Indeterminate result	Indeterminate result	0
9	No <i>Plasmodium</i> nucleic acid detected	No <i>Plasmodium</i> nucleic acid detected	2

2) For methods detecting *Plasmodium* only:

Positive specimens:

Code	Description	Report	Score
1	Genus and species correctly identified (e.g. <i>Plasmodium falciparum</i> nucleic acid present)	N/A	N/A
2	Correct genus but wrong species (incorrect <i>Plasmodium</i> species)	N/A	N/A
3	Only <i>Plasmodium</i> genus identified (e.g. <i>Plasmodium</i> spp. detected)	<i>Plasmodium</i> spp. detected	2
4	Indeterminate result	Indeterminate result	0
5	No <i>Plasmodium</i> nucleic acid detected	<i>Plasmodium</i> spp. not detected	-1
6	<i>Plasmodium falciparum</i> nucleic acid not detected (relevant for labs doing <i>Pf</i> identification only)	N/A	N/A

Negative specimens:

Code	Description	Report	Score
7	<i>Plasmodium</i> nucleic acid present	<i>Plasmodium</i> nucleic acid present	-1
8	Indeterminate result	Indeterminate result	0
9	No <i>Plasmodium</i> nucleic acid detected	No <i>Plasmodium</i> nucleic acid detected	2

3) For methods detecting *P. falciparum* only:

Positive specimens:

Code	Description	Report	Score
1	Genus and species correctly identified (e.g. <i>Plasmodium falciparum</i> nucleic acid present)	N/A	N/A
2	Correct genus but wrong species (incorrect <i>Plasmodium</i> species)	N/A	N/A
3	Only <i>Plasmodium</i> genus identified (e.g. <i>Plasmodium</i> spp. detected)	N/A	N/A
4	Indeterminate result	Indeterminate result	0
5	No <i>Plasmodium</i> nucleic acid detected	N/A	N/A
6	<i>Plasmodium falciparum</i> nucleic acid not detected (relevant for labs doing <i>Pf</i> identification only)	<i>P. falciparum</i> nucleic acid not detected	-1
7	<i>Plasmodium falciparum</i> nucleic acid detected (relevant for labs doing <i>Pf</i> identification only)	<i>P. falciparum</i> nucleic acid detected	2

Negative specimens:

Code	Description	Report	Score
7	<i>Plasmodium</i> nucleic acid present	<i>Plasmodium</i> nucleic acid present	N/A
8	Indeterminate result	Indeterminate result	0
9	No <i>Plasmodium</i> nucleic acid detected	No <i>Plasmodium</i> nucleic acid detected	N/A
10	<i>Plasmodium falciparum</i> nucleic acid not detected (relevant for labs doing <i>Pf</i> identification only)	<i>P. falciparum</i> nucleic acid not detected	2
11	<i>Plasmodium falciparum</i> nucleic acid detected (relevant for labs doing <i>Pf</i> identification only)	<i>P. falciparum</i> nucleic acid detected	-1

Appendix 4 – Letter of agreement

Letter of agreement

Dear Participant,

Re: External Quality Assurance Assessment Scheme for malaria nucleic acid amplification techniques

With a view to promoting the recent establishment by WHO of an international external quality assurance (EQA) scheme for malaria nucleic acid amplification techniques (NAAT), we have pleasure in inviting you to participate in the Scheme, which aims at strengthening and improving standards of performance of malaria NAAT in laboratories supporting malaria elimination through enhanced surveillance, case detection efforts and product development. Ultimately, improved performance in NAAT will ensure the policy making is based on the highest quality evidence, and interventions and resources are most appropriately targeted.

This Scheme aims at giving your laboratory the opportunity to measure its performance in nucleic acid amplification techniques for malaria through a confidential system of testing of blinded reference samples and to monitor its continuing performance.

The system is not aimed at delivering “good or bad marks” to participants nor to license laboratories. On the contrary, the Scheme is educational and aims at reinforcing mutual confidence within a network of laboratories. Participation is on a voluntary basis and will take place in a completely confidential setting.

WHO is collaborating with United Kingdom National External Quality Assessment Service (UK NEQAS) for Parasitology at Public Health England (PHE), as repository and distributor of the EQA materials, consisting of lyophilized and dried blood specimens of cultured and human malaria parasites, and parasite negative blood. UK NEQAS will also act as the operational center for the analysis and reporting of the results.

Participation is open to all interested laboratories up to the capacity of 65 laboratories. While participation is in principle open to all interested laboratories, if demand exceeds supply, rationing of participation will be done to generate a balance of laboratories according to testing indication and geography.

Fees will now apply for participation in the Scheme. The fees cover part of the costs incurred by WHO in the running of the Scheme.

For each analytical procedure, a participating laboratory will get a unique code number by which it will be identified in all routine transactions. It will furthermore receive a panel of ten lyophilized whole blood samples and dried blood spot samples, a set of instructions for use and handling, including storage precautions, a protocol and detailed information about the reporting of results through web-based electronic database. The material and documentation will be distributed by UK NEQAS, London in accordance with the instructions and directions of WHO.

Specimens distributed in the framework of the Scheme may contain fully virulent pathogens (other than hazard group 4, as classified by the Advisory Committee on Dangerous Pathogens, 1995 and the approved list of biological agents 2013). In this respect, they are identical to clinical specimens and must be handled, transported and stored with the same degree of caution and in compliance with all relevant laws, rules and regulations, including those applicable to the use of infectious substances and other biological materials. Samples must not be passed on to any third party. Samples are issued to participants on the understanding that they will be used exclusively for the purposes of this Scheme and that they will be handled by duly qualified staff. The samples are not designed for use in any other applications, e.g. for teaching, and participants are cautioned that their use for such other purposes may pose safety hazards.

Upon receipt, samples will need to be handled by the laboratory in accordance with the above-mentioned instructions and according to a quality assurance system appropriate to testing laboratories. Traceability must also be ensured. WHO cannot be held responsible for any damage to or loss of samples after customs clearance or alteration due to non-compliance with specific storage conditions.

Each round of proficiency testing typically takes approximately three weeks including the time between the dispatch by UK NEQAS and the return of results. This lengthy period is necessitated by the possibility of specimen shipment delays. Results returned after the closing date for return of results will be recorded as not returned.

Participants examine the specimens in their laboratories and report their findings to UK NEQAS by web forms (information about the service and your password will be sent to you following registration). Immediately after the closing date for return of results, brief details of the intended results are made available on the website. Replies are analysed and an individual report for each participating laboratory of its overall results for the distribution is placed on the website accessible to the laboratory concerned only through a secure password protected portal; participants are notified by email when the report has been posted on the website. Reports are normally available within 10 days of the closing date.

The individual report supplied after each distribution also provides laboratories with an individual analysis of their performance over a period of time (6 to 12 months depending on the distribution type). The following information is presented: i) a list of the specimens supplied during the period considered; ii) the number of results (if any) received too late for analysis; iii) the total score

achieved by the laboratory (derived by adding together the scores for each specimen); iv) the total possible score that would have been achieved with a fully correct result in all the specimens; v) the average score for the series.

Participation in the Scheme is subject to WHO and your laboratory (“the parties”) agreeing to treat any and all information, documentation and other material, exchanged as a result of or in connection with this Agreement, as strictly confidential. In this regard, each party shall take all reasonable measures to keep such information, documentation and other material confidential and shall use it only for the purpose of implementing the proficiency testing Scheme described in this letter. Thus, each party shall ensure that any persons having access to the said information, documentation and/or other material shall be made aware of and be required to adhere to like obligations of confidentiality and restrictions on use as contained herein. However, there shall be no such obligations of confidentiality or restrictions on use on a party, if and to the extent that the information, documentation and/or other material is publicly available at the time of signature of this letter or becomes thereafter publicly available through no fault of that party. In addition, the parties agree that WHO shall be entitled to make the reports of the proficiency tests publicly available in coded form.

Neither WHO nor UK NEQAS (including parties acting on behalf of WHO and UK NEQAS) shall accept any responsibility and/or liability whatsoever for the performance in proficiency testing described in this letter, the results of proficiency testing as reflected in the reports, any use made of, or follow-up given to, these reports, and/or the advice and recommendations contained therein or given in connection therewith. In this regard, each participating laboratory/you agree to hold WHO and UK NEQAS harmless from and against the full amount of any and all claims and liabilities, including legal fees and costs, which are or may be made, filed or assessed against WHO and/or UK NEQAS at any time, based on or arising out of the performance in proficiency testing and/or any use made of, or follow up given to, these reports and/or the advice and recommendations contained therein.

The results of the testing undertaken as part of this Scheme as contained in the reports provided by WHO to your laboratory, cannot in any way be construed as a certification or other endorsement by WHO and/or UK NEQAS, of the overall performance of your laboratory in the area of malaria nucleic acid amplification techniques or any other area. The aforesaid results, your participation in the Scheme or the WHO and/or UK NEQAS name and emblem cannot in any way be used by your laboratory or any other party for commercial and/or promotional purposes.

Any correspondence and other communications from your laboratory to WHO should be addressed to: malnaateqa@who.int

Any dispute relating to the interpretation of application of this letter will, unless amicably settled, be subject to conciliation. In the event of failure of the latter, the dispute will be settled by arbitration. The arbitration will be conducted in accordance with the modalities to be agreed upon by the parties or, in the absence of agreement, with the rules of arbitration of the International Chamber

of Commerce. The parties will accept the arbitral award as final. The arbitration shall take place in Geneva, Switzerland, unless the Parties agree otherwise.

Nothing in or relating to this letter shall be construed as an obligation on the part of WHO to submit to any national legislation or jurisdiction, and/or as a waiver of any of the privileges and immunities enjoyed by WHO under any national or international law, convention or agreement.

If your laboratory is interested in participating in this Scheme on the terms and conditions described in this letter, we should be grateful if you could arrange for a duly authorized representative of your laboratory to sign both copies of this letter and return one fully executed copy to WHO for its files. The duplicate copy is for your records.

In the hope that your laboratory will participate in this Scheme offered by WHO, we look forward to hearing from you and remain.

Yours sincerely,

Director, WHO Global Malaria Programme

Agreed and accepted on behalf of

[name of laboratory]

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Signature

Name

Title

Date

e-mail

Appendix 5 – Laboratory profile

Contact details														
Lab No	Title	First Name	Last Name	Job Title / Category	Phone	Mobile	Fax (if available)	Email	Organisation	Department	Country	Shipping Address	City	ZIP/Postal Code

Sample types processed in the laboratory											
lyophilized	DBS	LB									
		Genus lab is able to identify:					Species lab is able to identify:				
		Pf	Pv	Pk	Pm	Po	Pf	Pv	Pk	Pm	Po

										Valid import permit	Special shipment needs			
DBS														
Genus lab is able to identify:					Species lab is able to identify:						Include special documentation or details that should accompany the shipment.			
Pf	Pv	Pk	Pm	Po	Pf	Pv	Pk	Pm	Po					

Appendix 6 – Laboratory fees

Fee tier	Country classification	Lab type	Yearly fee (2 distributions) includes shipping costs
1	High income	Research	£800
2	High income	Public/ ref lab	£600
	Middle income	Research	£600
3	Middle income	Public/ ref lab	£300
	Low income	Research	£300
4	Low income	Public/ ref lab	£150