WHO external quality assurance scheme for malaria nucleic acid amplification testing

Operational Manual

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World Health Organization

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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].

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.

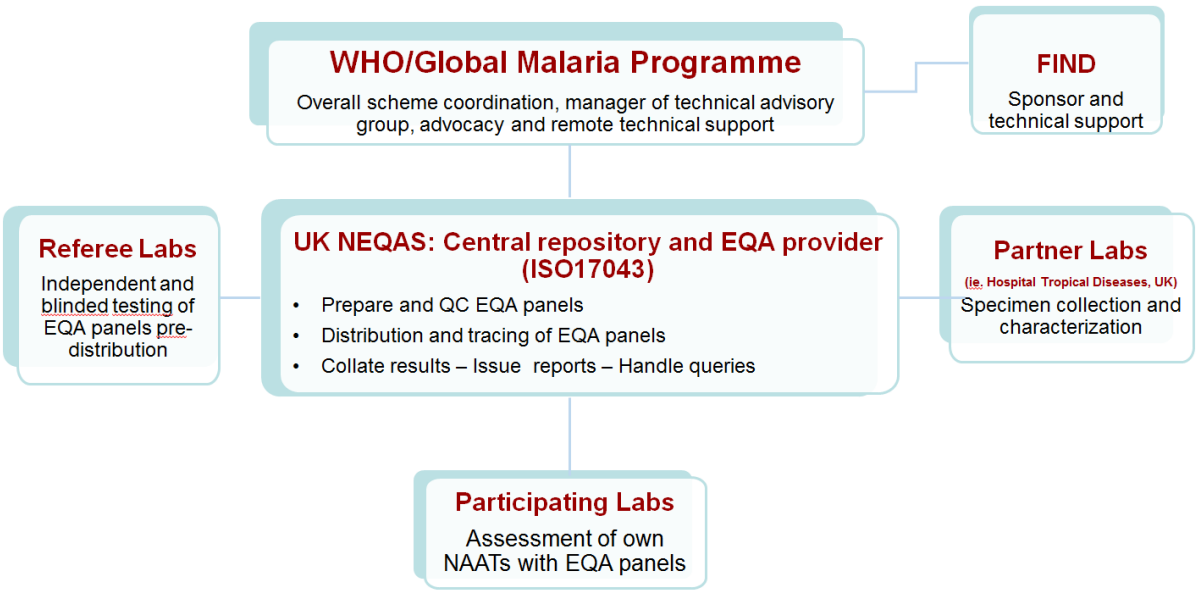
3 Objectives of this document

i. To outline the administration, functions and processes[1] 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), with support from the Foundation for Innovative New Diagnostics (FIND). 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. Currently, FIND provides financial support to the overall scheme and remote technical support to the participating laboratories, if required. 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@ukneqasmicro.org.uk . Preparation and characterization of materials is conducted at partner laboratories including the Hospital of Tropical Diseases (HTD), London.

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

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 (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.

An Advisory Group is convened on a regular basis (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 at the HTD and transferred to the UK NEQAS premises. *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 the National Health Service Blood and Transplant (NHSBT) that are confirmed to be parasite-negative by microscopy and nested PCR.

- Parasites from clinical samples