Malaria Policy Advisory Committee

Evidence Review Group (ERG) on diagnosis of *Plasmodium falciparum* in low transmission areas

Terms of Reference

Background

Light microscopy and antigen detecting, rapid diagnostic tests (RDTs) are the diagnostic tests recommended to guide the management of clinical malaria.[1] The use of malaria RDTs has particularly expanded to confirm suspected malaria cases in many malaria endemic countries.

For malaria population surveys, performed to monitor changes in malaria transmission, use of microscopy and/or RDTs underestimates the prevalence of low density parasite infections (<100parasites/µl). A systematic review of 42 published population surveys, comparing prevalence of *Plasmodium falciparum* based on light microscopy examination of blood slides with polymerase chain reaction (PCR)-based techniques, reported that the prevalence of infection by microscopy was, on average 50.8% of that measured by PCR.[2]. The review showed that under-estimation is even greater for gametocyte detection, and gametocyte rate measured by microscopy was on average 8.7% of the prevalence measured by PCR. A more recent review by the same authors showed that sub-microscopic malaria infections are more common in adults and in low endemic settings, and that when transmission reaches very low levels, submicroscopic carriers can be the source of 20-50% of all human-to mosquito transmission.[3] Overall, our understanding of the contribution of these low density submicroscopic infections to disease transmission is based on a limited number of studies.

Due to the limitations of microscopy and RDTs, increasingly, polymerase chain reaction (PCR)-based techniques which are several orders of magnitude more sensitive, are being applied for epidemiological surveys/studies, index case investigations, to analyse pre-patent parasitaemia in controlled human malaria infection (CHMI) trials, in drug efficacy trials and drug resistance research , as well as for the evaluation of new strategies/interventions aiming at transmission reduction (e.g. MDA, MSAT and FSAT).[4] Small subunit 18S ribosomal RNA (18SrRNA) molecular amplification, first exploited by Snounou et al [5] using a nested PCR technique is the most widely used molecular reference standard in malaria diagnostic research, and has been both adopted and adapted by many scientists. Beyond identification and speciation, there are quantitative methods using more sensitive real-time quantitative

1 WHO (2012). *Universal access to malaria diagnostic testing*. World Health Organization, Geneva.

2 Okell *et al.* (2009). Submicroscopic infection in *Plasmodium falciparum*-endemic populations: a systematic review and meta-analysis. *JID 2009 200:* 1509-1517.

3 Okell *et al.* (2012). Factors determining the occurrence of submicroscopic malaria infections and their relevance for control. *Nature Communications 3:127:* DOI: 10:1038/ncomms2241

4 Mass drug administration (MDA); Mass screening and treatment (MSAT); Focal screening and treatment (FSAT)

5 Snounou G, Viriyakosol S, Jarra W, Thaithong S, Brown KN: Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. *Molecular and Biochemical Parasitology* 1993: 58(2): 283-292.

PCR (qPCR) and nucleic acid sequence-based amplification (QT-NASBA) assays. More recently, a new commercial molecular assay based on loop-mediated isothermal amplification (LAMP) which uses simpler equipment and is less time-intensive may be a practical alternative to conventional PCR.[6] LAMP has been developed for the qualitative detection and species-specific identification of *Plasmodium* parasites using visual read-out and is independent of expensive thermal cyclers. Sensitivity is reported to approach that of nested PCR.[7] The LF-160 LAMP incubator (Eiken) accommodates 16 tubes/reactions per run and efforts are ongoing to develop a high-throughput LAMP based system, for broader use in population surveys and at lower costs.

The lack of clear consensus on standardized methods for qPCR makes it particularly difficult to interpret and compare results obtained by different research groups, and also to compare the performance of the different assays. In an effort to improve the consistency in publication of real-time PCR (qPCR), specific guidelines were developed in 2009 on Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE guidelines).[8] Only recently, using these guidelines, results of comparative performance of several qPCR assays have been published. [9] This initiative is particularly timely as the need for use of molecular based methods is mounting in countries in the malaria pre-elimination phase or in areas of containment of artemisinin resistance, despite demanding requirements for specialized laboratory equipment and high technical skills. WHO currently does not provide much needed guidance to countries regarding the programmatic suitability of molecular diagnostics and how to utilize the information that would emerge from their use. Microscopy is still officially considered the gold standard despite large bodies of evidence to support PCR as the gold standard for detection of malaria infection. Furthermore, where these techniques may be suited, guidance on indications for use, assay selection and quality assurance/control must also be developed.

To this end, WHO/GMP is proposing to the Malaria Policy Advisory Committee to establish an Evidence Review Group (ERG) on diagnosis of *P. falciparum* in low transmission areas on 16-18 December 2013 that would review a) the burden of submicroscopic malaria parasitaemia; b) the evidence of its contribution to disease transmission; c) molecular diagnostics testing options (and targets); and d) their comparative performance and programmatic suitability. Furthermore the ERG would review current WHO recommendations on exclusive use of light microscopy for confirmation of malaria species and detection of gametocytes in the advanced programmatic phases of malaria elimination. WHO will also collaborate with PATH's DIAMETER[10] project to present ERG members with an overview of the malaria diagnostic R&D pipeline and to review target product profiles developed to address needs of various scenarios[11] for malaria elimination.

6 Hopkins et al J Infect Dis. 2013 Aug;208(4):645-52.

7 Polley S.D. et al. J Infect Dis. 2013 Aug;208(4):637-44.

8 Bustin *et al.* (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry 55:* 611-622.

9 Alemayehu *et al.* (2013). Comparative evaluation of published real-time PCR assays for the detection of malaria following MIQE quidelines. *Malaria Journal* 12:277

10 DIAMETER – Diagnostics for Malaria Elimination Toward Eradication

11 Passive case detection and active infection detection strategies such as reactive case detection (RACD); screening and treatment programs; population surveys; and laboratory analysis for infection confirmation, origin determination, and identification of drug resistance.

WHO/GMP secretariat is proposing that the ERG will be convened to develop draft recommendations on the diagnosis of *P. falciparum* in low transmission settings for review and endorsement by the MPAC in March 2014.

Specific Objectives

1. Review knowledge on prevalence of submicroscopic parasitemia in asymptomatic populations in low, moderate and high transmission settings.

2. Review evidence on the contribution of asymptomatic parasitemia to transmission, with special emphasis on areas with low transmission.

3. Review diagnostic performance of available molecular based methods (and targets) for diagnosing low density infections with sexual and asexual malaria parasites.

4. Compare and contrast the technical and resource requirements of existing malaria molecular methods most suitable for use in population surveys and active case detection among asymptomatic people in low transmission settings.

5. Review the evidence base for current WHO recommendations for malaria diagnosis in pre-elimination and elimination settings.

6. Define the potential role of malaria RDTs in addition to microscopy in pre-elimination and elimination settings.

7. Review the diagnostic R&D pipeline for diagnosis of low density malaria infection

8. Reach consensus on the preferred product characteristics of new diagnostic tools to meet public health needs in malaria elimination settings.

9. Compile ideas on how to build capacity and ensure quality for molecular diagnostic methods for use in active case detection, population surveys and malaria surveillance in pre elimination and elimination settings.

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