Proposed Evidence Review Group on malaria submicroscopic infections

Malaria Policy Advisory Committee (MPAC) Meeting
22-24 March 2017, World Health Organization, Geneva, Switzerland
Presentation Outline

• Background
• WHO recommendations on malaria diagnostics
• Justification for new ERG on submicroscopic malaria infections
• Proposed objectives of ERG
• Discussion
Recommendations on malaria diagnostics

WHO recommendation 1

- Quality assured RDT and microscopy are the primary diagnostic tools for the confirmation and management of suspected clinical malaria in all epidemiological situations, including areas of low transmission, due to their high diagnostic performance in detecting clinical malaria, their wide availability and relatively low cost. Similarly, RDT and microscopy are appropriate tools for routine malaria surveillance (of clinical cases) in the majority of malaria-endemic settings.

Recommendation 2...6 (related to Nucleic Acid Amplification Techniques)
Detecting sub-microscopic infections

- Who are we missing with microscopy and RDTs?
- What factors influence submicroscopic infections?
- What are their contribution to transmission?
- When and how to target them?
The prevalence of infection measured by microscopy was, on average, 54.1% of that measured by PCR. Submicroscopic parasite carriage more common in adults.

The gametocyte rate measured by microscopy was, on average, 8.7% of that measured by PCR.


Okell et al. Nature Communications (2012) DOI: 10.1038/ncomms2241
WHO current recommendations (cont'd)

• A number of NAA techniques are available and are more sensitive in detection of malaria compared to RDTs and microscopy. Generally, the use of more sensitive diagnostic tools should be considered only in low transmission settings where there is already widespread implementation of malaria diagnostic testing and treatment and low parasite prevalence rates (e.g. < 10%). Use of NAA-based methods should not divert resources away from malaria prevention and control interventions and strengthening of the health care services, including the surveillance system.

• Submicroscopic *Plasmodium falciparum* and *Plasmodium vivax* infections are common in low as well as in high transmission settings. The use of NAA methods by malaria programs should be considered for epidemiological research and surveys aimed at mapping submicroscopic infections at low transmission intensity. There may also be a use for NAA methods for identifying foci for special intervention measures in elimination settings.
The majority of infections with asexual parasites have gametocytes detectable by molecular amplification methods, at low density not detectable by microscopy or RDTs. Most malaria infections (microscopic and submicroscopic) should be considered as potentially infectious and able to contribute to ongoing transmission. **There is no need for routine detection of gametocytes using sensitive mRNA amplification methods in malaria surveys or clinical settings.**

**Infection = infectious / soon to be infectious**

_Bousema & Drakeley, Clin Micr Rev 2011_
WHO current recommendations (cont'd)

• Common standards for nucleic acid based assays should be developed, including use of the WHO International *P. falciparum* DNA Standard for NAA assays and development of standards for other *Plasmodium* species, particularly *P. vivax* should be undertaken. A standard operating procedure should be developed which defines methods for sample collection, extraction, and the recommended equivalent quantity of blood to be added to the assay. Development of an international, external quality assurance system is strongly recommended to ensure that data obtained from NAAs are reliable and comparable.

• In order to establish the role of serological assays in epidemiological assessments, there is a need for standardization and validation of reagents (antigens and controls), assay methodologies and analytical approaches.
## NAA methods to detect low parasitaemia

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<th>Diagnostic technique</th>
<th>Operational characteristics</th>
<th>Performance&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Cost&lt;sup&gt;2&lt;/sup&gt;</th>
<th>References</th>
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<tr>
<td><strong>Nested PCR</strong></td>
<td>Uses two sets of primers in successive reactions, therefore increased cost, time and potential for contamination compared to single step PCR.</td>
<td>Limit of detection of at least 6 p/μl for blood spots. Higher sensitivity than single step PCR for four major <em>Plasmodium</em> species. Hands-on time 3 hours to result, total time 10 hours.</td>
<td>$1.5-4.0 per sample, $500-5000 for equipment</td>
<td>[24]</td>
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<tr>
<td><strong>Multiplexed PCR</strong></td>
<td>Simultaneous, multiplex PCR to detect the presence of multiple <em>Plasmodium</em> species.</td>
<td>Limit of detection 0.2-5 p/μl. 2 hours hands-on time to result, total time 4.5 hours.</td>
<td>$1.5-4.0 per sample (but lower than nested), $500-5000 for equipment</td>
<td>[25-28]</td>
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<tr>
<td><strong>Quantitative PCR</strong></td>
<td>Rapid amplification, simultaneous detection and quantification of target DNA through use of specific fluorophore probes.</td>
<td>Limit of detection 0.02 p/μl for genus level identification, 1.22 p/μl for <em>P. falciparum</em> detection. 60 minutes hands-on time to result, total time 2.5 hours.</td>
<td>$4-5 per sample, &gt;$20,000 for equipment</td>
<td>[29-32]</td>
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<td><strong>LAMP</strong></td>
<td>Boil and spin extraction can be used, amplification by isothermal method. Result determined by turbidity or fluorescence. Sensitivity can be increased by including mitochondrial targets. Genus level targets, <em>P. falciparum</em> and <em>P. vivax</em>. Field-appropriate.</td>
<td>Limit of detection 0.2-2 p/μl. Results can be available in 30 minutes with a tube scanner.</td>
<td>$4-5 per sample (commercial), $500-5000 for equipment</td>
<td>[33-37]</td>
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<td><strong>QT-NASBA</strong></td>
<td>Assay includes a reverse transcriptase step, less inhibition than PCR. Isothermal method. Can be used for gametocyte quantification. Detects all four <em>Plasmodium</em> species, targeting 18S rRNA. Result by fluorescence.</td>
<td>Limit of detection 0.01-0.1 p/μl per 50μl sample. 90 minutes for result (not including extraction time of an additional ~90 minutes)</td>
<td>$5-20 per sample. ? equipment costs</td>
<td>[38-40]</td>
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<sup>1</sup> Diagnostic performance influenced by factors including sample preparation, NA extraction efficiency, and amount of blood, amount of template included in reaction, copy number of target sequence, and specific buffers, enzymes etc. used.

Malaria diagnostics in low transmission

**Routine surveillance and passive case detection:**
- Based on appropriate case definition of suspected malaria, microscopy and RDTs are sufficient.

**Malaria epidemiological surveys:**
- Molecular test (or other technology) with analytical sensitivity of ~2 parasites/µl to detect the substantial proportion of low density infections (e.g. classic PCR, qPCR and LAMP or other tests with similar LOD).
- Rapid turnaround is not a priority; internal and external QA is required.

**Foci investigations:**
- A molecular test (or other technology) with analytical sensitivity of ~ 2 parasites/µl.
- Turn-around time should be <48 hours to allow prompt follow up and treatment of positive individuals; internal and external QA is required.
Malaria diagnostics in low transmission

**Mass screening and treatment:**
- RDT and microscopy are not sufficiently sensitive
- Molecular test (or other technology) with moderate throughput and analytical sensitivity of ~2 parasites/µl to detect low density infections.
- Results ideally on the same day to maximise follow up and treatment of positive individuals; internal and external QA is required.

**Screening of special populations (e.g. at border crossings):**
- RDT or microscopy should be used for symptomatic infections only.
- Molecular tests with analytical sensitivity of 2 parasites/µl should be used for detection of infection in asymptomatic individuals.
- Results should be provided on the same day to minimize loss to follow-up.

To be a "significant improvement" over expert microscopy, molecular (and non-molecular) methods needs to be at least one log more sensitive than microscopy i.e. able to detect 2 parasites/µl or fewer.
WHO policy brief on malaria diagnostics

The complete policy brief is available on WHO webpage at the following link:

http://www.who.int/entity/malaria/publications/atoz/malaria-diagnosis-low-transmission-settings-sep2014.pdf?ua=1
The 2013 ERG did not conduct a detailed assessment of the natural history of submicroscopic infections and their epidemiological contribution to transmission, nor did it provide recommendations on different detection/screening/surveillance approaches and how to utilize the information emerging from their use.

In recent years, the application of NAA-based diagnostic tools in epidemiological surveys and research has continued to expand.

More recently, funding agencies, manufacturers and researchers have been working towards developing ultra-sensitive RDTs with limits of detection similar to those of NAA-based methods.
WHO guidance for malaria elimination

• RDTs and microscopy can be used to detect almost all symptomatic infections and many but not all asymptomatic infections.

• More sensitive diagnostic methods, such as polymerase chain reaction and other molecular techniques, are used to detect asymptomatic infections with very low parasite densities.

• These tests may be useful in surveys for mapping submicroscopic infections, but their value depends on the epidemiological significance of low-density infections, which is not yet sufficiently defined.

• If local malaria transmission persists despite intensive vector control and universally good case management, the programme may consider undertaking special studies to evaluate the distribution and frequency of infections in the asymptomatic population.
Absence of Asymptomatic Malaria Infections in Previously High Endemic Areas of Sri Lanka.

Pre-elimination stage of malaria in Sri Lanka: assessing the level of hidden parasites in the population
Rajakaruna et al. Malaria Journal 2010, 9:25
Proposed objectives of the ERG

1. To review data on the natural history of submicroscopic *P. falciparum* and *P. vivax* infections in different epidemiological settings, and implications for detectability, duration of infection, and infectivity, and the relationship with symptoms of clinical malaria.

2. To describe at population level the contribution of submicroscopic *P. falciparum* and *P. vivax* infections to transmission with respect to different levels of vectorial capacity and immunity in the population.

3. To define procedures for the case management and reporting of submicroscopic *P. falciparum* and *P. vivax* infections identified through multiple means, e.g., reactive case detection, surveys, research, etc.
Proposed objectives of the ERG

4. To review and **update the WHO recommendations** on the diagnosis of *P. falciparum* and *P. vivax* malaria in low transmission settings, which were endorsed by the Malaria Policy Advisory Committee in March 2014, based on the report of the 2013 ERG meeting.

   - Submicroscopic *Plasmodium falciparum* and *Plasmodium vivax* infections are common in low as well as in high transmission settings. The use of NAA methods by malaria programs should be considered for epidemiological research and surveys aimed at mapping submicroscopic infections at low transmission intensity. There may also be a use for NAA methods for identifying foci for special intervention measures in elimination settings.

5. To establish a set of **research priorities** and study design characteristics to address knowledge gaps on the relative importance of submicroscopic infections and the public health impact of detecting them using highly sensitive diagnostic tests.