Evidence Review Group on Malaria Diagnostics in Low Transmission Settings: meeting report and draft recommendations for MPAC consideration

ERG meeting held in Geneva, Switzerland
16-18 December 2013

MPAC meeting
WHO HQ, 14 March 2014
Participants

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**Meeting procedures described on page 4 of the Report**
Meeting objectives

P. falciparum & P. vivax

• Review current knowledge on contribution of asymptomatic parasitaemia to transmission, particularly in areas with low transmission

• Review diagnostic performance, technical and resource requirements of available nucleic acid amplification (NAA) methods for diagnosing low density infections with sexual and asexual malaria parasites; recommend most suitable methods for use in population surveys and active or reactive investigations

• Review requirements to build capacity and ensure quality for NAA methods to support programmatic interventions in pre-elimination and elimination settings

• Review and suggest revisions to current WHO recommendations for malaria diagnostic approaches in low transmission settings

• Review malaria diagnostic R&D pipeline; reach consensus on preferred product characteristics of new diagnostic tools to meet public health needs for malaria elimination
Focus is **NOT** High/moderate transmission settings or role of NAA based tests for detection of clinical cases.

**Settings characterized by:**
- Lower incidence of confirmed cases
- More uniform spread by age or concentrated in population groups with higher exposure / focal within districts
- Lower mortality rates
- Parasite prevalence (2-9yrs): <10%

**High coverage of interventions:**
- diagnostic testing
- LLIN, IRS

**Surveillance system with good reporting rates in place**

**Table 1: Malaria surveillance in different transmission settings and phases of control**

<table>
<thead>
<tr>
<th>Transmission:</th>
<th>Control phase</th>
<th>Elimination phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>High &amp; moderate</td>
<td>Low</td>
<td>Very low</td>
</tr>
<tr>
<td>Parasite prevalence (2-9 yrs):</td>
<td>&gt;1%</td>
<td>&lt;10%</td>
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</table>

**Incidence:**
- Cases and deaths common and concentrated in <5yrs
- Limited temporal variation
- Limited geographical variation

- Cases and deaths less common distributed according to mosquito bite exposure
- Variable within and between years
- Risk of epidemics
- Geographical heterogeneity
- Concentrated in marginal populations

- Cases sporadic
- Imported cases may be high proportion of total
- Focal distribution

**Fevers:**
- Proportion of fevers due to malaria relatively large
- High proportion due to malaria
- Efficient
- Mortality & case reduction

- Proportion of fevers due to malaria small
- Low proportion due to malaria
- Controlled efficient/inefficient
- Controlled efficient/inefficient

**Health facility attendance:**
- Resources:
  - Low expenditures per head
  - Low quality and poor accessibility of services

- Data recording:
  - Aggregate numbers

- Investigation:
  - Inpatient cases

**Surveillance system:**
- Widespread availability of diagnostics and treatment
- Aggregate numbers
- Lists of inpatients and deaths
- Inpatient cases → all cases

Who are we missing with microscopy and RDTs?
What factors influence the asymptomatic reservoir?
What is its contribution to transmission?
When and how to target it?
Targeting the asymptomatic reservoir

• Multiple survey approaches utilize diagnostic testing
  – Prevalence surveys, focal screen and treat (FSAT), mass screen and treat (MSAT), highly focused screening and treatment (HiFSAT) – this ERG meeting did NOT analyse and recommend specific survey approaches vs others

• Diagnostic tools for infection detection
  – Microscopy, RDTs, nucleic acid based methods
  – Sexual vs asexual parasites; hypnozoites
  – Considerations: ‘required’ limit of detection for public health impact, quantitative vs non quantitative, programmatic suitability, cost, and quality control
  – Role of serology

• Knowledge gaps and research needs
What are the recommended tests to detect asymptomatic infections in population surveys, active case detection, screening, and case management in elimination settings?

What is the gold standard of malaria diagnosis in elimination setting?

What are the recommended diagnostic tools to be used at community level in areas targeted for malaria elimination, considering the limitations of microscopy and RDTs?

What is the role of PCR in malaria elimination settings for surveillance and case management, and what are the requirements for quality assurance?

What are the most sensitive and easy to use assays to detect gametocytes and their contribution to transmission, for use in research studies?

What is the best screening tool for detection of malaria asymptomatic carriers in airports and at borders?

Can current serological tests (ELISA) assist in differentiating recent versus old infection?

What are the best diagnostic tools to confirm interruption of transmission, for certification of malaria elimination?

What resources and tools are required to sustain diagnosis capacity in low transmission settings and/or in areas at risk of re-introduction of malaria?

ANNEX 1 of the Report (pages 23-24)
Inform/update current recommendations/guidance

ANNEX 2 of the Report (pages 25-29)
Draft Recommendations

• 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.
Review of 94 unique eligible studies comparing RDTs with microscopy:

- For HRP-2, the meta-analytical average sensitivity and specificity (95% CI) were 95.0% (93.5% to 96.2%) and 95.2% (93.4% to 99.4%), respectively.

- For pLDH, the meta-analytical average sensitivity and specificity (95% CI) were 93.2% (88.0% to 96.2%) and 98.5% (96.7% to 99.4%), respectively.

• 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 nucleic acid amplification (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 P. vivax infections are common in low as well as high transmission settings. A number of nucleic acid amplification techniques are available and are more sensitive in detection of malaria compared to RDTs and microscopy. The use of NAA methods by malaria programs should be considered for surveys aimed at mapping prevalence of malaria, including submicroscopic infections, and to increase the power of surveys at low transmission intensity.
A low percentage of total infections is detected by microscopy in areas of low transmission, compared with areas at high transmission, (12.0% when at PCR prevalence was <10% versus 74.5% at PCR prevalence >75%).
Submicroscopic *P. falciparum* infection

- 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 C, Ghani A, Lyons, E et al. JID 2009: 200: 1509-17*
• The majority of infections with asexual parasites have gametocytes detectable by molecular amplification methods. All malaria infections (microscopic and submicroscopic) should be considered as potentially infectious and able to contribute to ongoing transmission. There is no operational need for routine detection of gametocytes in malaria surveys. For research applications, nucleic acid sequence-based amplification (i.e. QT-NASBA or real time qPCR) are the recommended gametocyte detection tools.
The need to detect gametocytes

• YES:
  • Transmission reducing interventions
  • Assessing the human infectious reservoir
  • Understanding the dynamics of infections

• NO:
  • community surveys for interventions
  • molecular tools have done their work

• Infection = infectious / soon to be infectious

Courtesy of: Teun Bousema, Lucy Okell, Andre Lin Ouedraogo, Chris Drakeley

Bousema & Drakeley, Clin Micr Rev 2011
Detecting submicroscopic gametocytes

Light Microscopy:
- very limited sensitivity

qRT-PCR:
- Pfs25 is most abundant target

Sampling strategies:
- RNA protective buffers
- Filter papers stored at -80
- Filter papers stored at -20
- Not in saliva (18S possible)
- Not in urine (18S possible)

Courtesy of: Teun Bousema, Lucy Okell, Andre Lin Ouedraogo, Chris Drakeley

Felger, unpublished data
Wampfler et al. PLoS ONE 2013
Jones et al. Malar J 2012
• Common standards for nucleic acid based assays should be developed, including use of the WHO International Standard for P. falciparum DNA NAT 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 nucleic acid amplification assays are reliable and comparable.
The Minimum Information for Publication of Quantitative Real-Time Experiments: The MIQE Guidelines

• “Lack of consensus on how to perform qPCR experiments”
  – Possible quantification calibrators:
    • Synthetic RNA or DNA oligonucleotides
    • Plasmid DNA constructs
    • cDNA cloned into plasmids
    • RNA transcribed in vitro
    • Reference RNA pools
    • RNA or DNA from specific biological samples
    • Internationally recognized biological standards

• Comparative evaluation of 7 published real-time PCR assays for the detection of malaria following MIQE guidelines
Alemayehu et al. Malaria Journal 2013, 12:277
  – 7 published qPCR assays detecting Plasmodium spp or P.falciparum compared using standard DNA and samples from a clinical trial: 6/7 assays showed sensitivity lower than what have been published.
Proposed development of an international, external quality assurance system for NAT assays

Central Repository to Manage Scheme
- Source and characterise specimens
- Pre- and post-distribution checks
  - Collate results
  - Issue reports
  - Handle queries

Partner Labs
- Specimen characterization

Regional Hubs

Referee Labs
- On rotation

National Schemes
- Maintain concordance with global scheme
- Distribute extra material relevant locally

Individual Labs

Distribution of calibrators

Courtesy of Peter Chiodini
• There is a need for standardisation of reagents (antigens and controls), assay methodologies and analysis for malaria serology. Until that becomes available there is a limited role for serological assays in the routine operational monitoring of transmission in elimination settings, but they may still have a role for epidemiological surveys in certain elimination settings.
Uncertainty over contribution of transmission, i.e. estimated submicroscopic parasitaemia source of 20% mosquito infections when malaria slide prevalence is:

- < 0.5% (Young et al., 1948)
- < 4% (Jeffery and Eyles., 1955)
- < 24% (ALO, unpublished data)

*Estimated by Okell C, et al. NC 2012: 3:1237 DOI: 10.1038/ncomms2241
There is a need for more research to understand better the contribution of submicroscopic infections in malaria transmission in low endemic settings and to identify which diagnostic strategies and NAA-based diagnostic techniques are most cost-effective in accelerating malaria elimination, compared to conventional malaria elimination methods. Additionally, markers to identify recent malaria infections, and diagnostic tools that detect P. vivax hypnozoites are needed.
<table>
<thead>
<tr>
<th>Diagnostic technique</th>
<th>Operational characteristics</th>
<th>Performance¹</th>
<th>Cost²</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nested PCR</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>
</tr>
<tr>
<td>Multiplexed PCR</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>
</tr>
<tr>
<td>Quantitative PCR</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>
</tr>
<tr>
<td>LAMP</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>
</tr>
<tr>
<td>QT-NASBA</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>
</tr>
</tbody>
</table>

¹ 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.

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.
Applications of malaria diagnostics in low transmission settings (ii)

**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 minimise 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.
Summary of key points

• Quality assured RDT and microscopy are the primary diagnostic tools for clinical management of malaria in all epidemiological situations, and for routine malaria surveillance in most malaria-endemic settings.

• Submicroscopic *P. falciparum* and *P. vivax* infections are common in low as well as high transmission settings. The majority of infections with asexual parasites have gametocytes detectable by molecular amplification methods, and do contribute to malaria transmission.

• The use of NAA methods by malaria programs should be considered for epidemiological surveys aimed at mapping prevalence of malaria, foci investigations, mass screening and treatment strategies, and screening of special groups (e.g. border screening) when the aim is also to detect submicroscopic infections.

• Molecular tests with analytical sensitivity of 2 parasites/µl should be used for detection of infection in asymptomatic individuals.

• Development of an international, external quality assurance system is strongly recommended to ensure that data obtained from nucleic acid amplification assays are reliable and comparable.