Malaria diagnostics: Progress, possibilities and priorities

3rd Manipal International Infectious Diseases Conference 2019
Theme: Tropical Infections and Global Health

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Malaria distribution and progress since 2000 – P. falciparum

https://map.ox.ac.uk/
Malaria distribution and progress since 2000 – *P. vivax*

- **Less common:**
  - *P. ovale* (curtisi, wallikeri)
  - *P. malariae*
  - *P. knowlesi*

https://map.ox.ac.uk/

48% in India (World Malaria Report 2018)
But progress is slowed, or halted... Global malaria trends 2010 - 2017

Global malaria trends: cases per 1000, deaths per 100K of pop’n at risk

Trends in malaria case incidence rate (cases per 1000 population at risk), globally and by WHO region, 2010–2017. The WHO European Region has reported zero indigenous cases since 2015. Source: WHO estimates.


Countries with large changes in malaria case rate

Number of countries and areas where a reduction (green) or an increase (red) of more than 20% in malaria cases has occurred between 2016 and 2017, by WHO region. Sources: NMCP reports and WHO estimates.
...And funding is fragile...

Malaria diagnosis – Why?

**Diagnosis of disease**
- Distinguish malaria-related fever from other causes:
  - Direct use of anti-malarial drugs
  - Identify non-malaria cases requiring further investigation

**Gather data on malaria incidence**
- Planning and targeting of interventions (assumes a decent HIS)

**Detection of parasites**
- Parasite-screening tests
  - Detect parasitaemia for treatment, to prevent onward transmission

**Detection of transmission**
- Population screening tests
Malaria detection and malaria prevalence

- Holo-endemic
- Elimination

- Case management (Microscopy or RDTs)
- Surveillance /surveys
- Screening (asymptomatics)
- Screening (borders)
- Drug efficacy monitoring
Why distinguish between these classes of diagnostics?

Diagnosis of disease, Parasite detection

Implications:

- Different product specifications
- Different implementation challenges
- Different funders /funding mechanisms
- Different willingness-to-pay
Current tests for case management
Light microscopy

~200M diagnoses /year
Quantify parasites
Species differentiation
Useful for other diseases...

But...

Highly technician dependent
Highly variable threshold of detection
Not so cheap to do it well
*Slow for Point-of-Care*

Go digital
Routine microscopy


<table>
<thead>
<tr>
<th>Hospital</th>
<th>Routine microscopy</th>
<th>Expert microscopy</th>
<th>RDT (other patients)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>10</td>
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<td>5</td>
<td>2</td>
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<td>Disp 6</td>
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<td>3</td>
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</tbody>
</table>
Factors influencing microscopy quality

Selection

Training

Assessment

Competency

Supervision/ Cross-checking

Equipment/ reagents

Support network/ Slide results delivery

Work environment/ accuracy

Performance/ accuracy

Good microscopy is not cheap!
Commercially-available Rapid Diagnostic Tests

~300M diagnoses /year

Target antigens

Histidine-rich protein 2 (HRP2)
  P. falciparum only
  Antigen persists in circulation after treatment

Plasmodium Lactate dehydrogenase (pLDH)
  Pan/specific or species-specific
  Clears rapidly after treatment

(Aldolase)

Formats:
  Pf-only
  Pf/pan
  Pf/Pv
  Other
Maintaining diagnostic quality

Supply chain management

Transport and storage

End users
- Appropriate training and instructions
- Management of positive and negative results
- Monitoring of commodity supply and disease rates

Stage 1: Pre-qualification / Product testing
Evaluate product performance, manufacture quality

Stage 2: Testing manufactured lots within country (India - NIMR)
Confirm product quality on arrival.
Test samples from field

Manufacture
- ISO13485 etc
WHO Malaria RDT Product Testing: Rounds 1-8 results (227 unique products)

P. falciparum

https://apps.who.int/iris/bitstream/handle/10665/276193/9789241514958-eng.pdf?ua=1
Are these tests sensitive enough?

Is it safe to **withhold anti-malarial treatment** from RDT-negative febrile children in high-prevalence *P. falciparum* areas?

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Population</th>
<th>RDT Results</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>D’Acremont V., <em>Clin Infect Dis</em> 2010. Tanzania (ParaHit f)</td>
<td>1000 children with fever, 601 RDT negative, no antimalarials</td>
<td>Mortality: 2 (non-malarial causes)</td>
<td></td>
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</tr>
<tr>
<td>Mtve G et al. <em>Malar J</em> 2011. Tanzania (ParaHit f)</td>
<td>965 children with fever, 807 RDT negative, no antimalarials (~various other)</td>
<td>No mortality</td>
<td></td>
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</tr>
</tbody>
</table>
WHO Malaria RDT Product Testing: Rounds 1-8 results (227 unique products)

*P.* vivax

Cochrane review of RDT field performance on non-falciparum parasites, 2014 (currently being updated):

**Versus microscopy:**
- **Sensitivity** 78-89%
- **Specificity** 98-100%
Translating lab performance into clinical performance
Impact of commercial pressures and realities of routine logistics and use.
Improving user performance

Zambia MoH, URC, WHO, TDR, FIND, Malaria Consortium
Case management - Threats and challenges.....
Threats and challenges.....

Vivax management

- *P. vivax* tests remain insufficiently sensitive
- No tests exist for liver stage (latent) *P. vivax* infection
- 14 day courses of *primaquine* (liver stage clearance) are poorly adhered to.
- Therefore, reduction in transmission is greatly retarded by relapse
- *Tafenoquine* (single dose 8-aminoquinolone) now prequalified but requires G6PD deficiency testing (*single long-acting dose, so ~higher risk of severe haemolysis*)
  
  …but no good point-of-care G6PD test exist
- We will likely not accelerate *P. vivax* elimination until part or all of this is solved.
Threats and challenges.....

HRP2 deletions

HRP2 (& HRP3) deletion noted ~ 2007. Evidence of genesis up to 30 years earlier


https://map.ox.ac.uk
Parasitaemia in very low transmission /elimination
How sensitive do we need to be?

Tradeoff between sensitivity and ability to implement

Place of POC diagnostic methods in malaria detection...

May be possible to transmit infection through all of this period

Derived from observations of controlled infections to induce fever in tertiary syphilis patients, compiled by Collins and Jeffreys, US CDC. Unpublished data.
Focusing screening and treatment (FSAT) in areas with passively-detected foci

robust anthropophilic vector (e.g. A. gambiae, A. farauti)

1. Passive Surveillance: Malaria cases presenting at health centres over a few months

2. Screen surrounding houses within # radius with very sensitive test to detect asymptomatic cases

Much less likely to transmit
Focal screening (FSAT) around a confined vector breeding site

(A flavirostris: Foley et al. TRSTMH, 2003)

1. Population screening: Broad survey of #% of population, identifies foci (cluster) of recently-infected people

2. Screen surrounding houses within # radius of known breeding site, with very sensitive test to detect asymptomatic cases
Extending the window of detection of ‘infection’ to identify transmission foci within a broad area

Derived from observations of controlled infections to induce fever in tertiary syphilis patients, compiled by Collins and Jeffreys, US CDC. Unpublished data.
1. Population screening: Broad survey of % of population, identifies foci (cluster) of recently-infected people

2. Screen surrounding houses within # radius with very sensitive test to detect asymptomatic cases

Identifying and managing foci through broad population surveys
Emerging technologies
Detecting very low density infection – Current options

**Nucleic acid amplification tests (NAAT):**

**PCR....** Potentially cassette-based field products in future  
Current PCR techniques applicable if lab close to community (e.g., Cambodia)

**LAMP...** Capable of near-patient operation with sensitivity similar to PCR

**Enhancement of Lateral Flow Assays (RDT)**

- Improved LFA strip  
- Sample pre-concentration  
- Signal enhancement with e-readers

**Other:**

- Haemozoin detection  
- Digital microscopy, pattern-recognition software,  
- All those cell-phone Apps...
LAMP
(Loop-mediated isothermal amplification)
Malaria LAMP Assay (Eiken /FIND):
- Need heat block, +/- centrifuge, reaction tubes, bench.
- Results (including sample prep) within 2 hours
- Reading: visual (fluorescence) or turbidimeter

-Sensitivity roughly equivalent to PCR
-98% sensitivity at ≥ 2 para/µL
  * Hopkins et al. JID, 2013, Sutherland et al. JID, 2013

Fresh samples, dry blood spots
Much simper, nearer patient than PCR
Cassette-based PCR

**e.g. QuantumDx:**
- Cassette-based PCR
- ~20 minutes to result
- Disposable cassette in Reader module
- Company was initially concentrating on malaria diagnosis
  - Now pre-cancer, STI etc

*Standardizing PCR and reducing costs remains a major issue for malaria research and elimination, but who would pay?*

_Courtesy of QuantumDx_
Enhancement of Lateral Flow Assays (RDT)

e.g. Abbott (Alere) Ultra-sensitive HRP2 RDT
  - Some improvement on current RDTs (lower limit of detection)
    • Outcomes vary between different studies
  - 20 minutes to result (as per case-management RDTs)
  - *P. falciparum* only

Various groups developing more sensitive RDTs for *P. vivax* (LDH)

Serology tests for *P. falciparum* and *P. vivax* transmission detection in development

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Haemozoin detection

e.g. Hemex Gazelle malaria assay
- Detects haemozoin in finger-prick blood
- 1 minute from sampling to result
- Similar sensitivity to current RDTs for *P. falciparum*
- Higher sensitivity for *P. vivax*
- High specificity

- **Under trial at Manipal**

- **Uses:**
  - ? Vivax diagnosis
  - HRP2-deleted *P. falciparum*
  - Rapid screening
  - ?

Various groups evaluating potential for non-invasive Hz detection
Summary

Case management

• Good tools for *P. falciparum*
• May need to re-think vivax diagnosis if to make significant inroads
• But much actual malaria diagnosis remains symptom-based, with parasite-based diagnosis mainly supported by external funding (*highly fragile*)

Elimination

• Screening tools are *inadequate*, need:
  – Better management systems for routine diagnostic data
  – More sensitive screening tests
  – Population screening tests (monitor transmission)
    ...that are affordable and usable within national programs (i.e. sustainable)

Global market for elimination tests remains highly dependent on mood of external funders – not a good prospect for manufacturers
Thank you