WHO-FIND Malaria RDT Evaluation Programme: Product Testing Round 5

Malaria Policy Advisory Committee Meeting
WHO HQ Geneva, 11 September 2014

Jane Cunningham
Global Malaria Programme

World Health Organization

GLOBAL MALARIA PROGRAMME
Overview

• Background
• Overview of Product testing process
• Round 5 results – what’s new?
• WHO procurement criteria
• Market trends and impact on manufacturers
• Future
  – Product testing and lot testing based on recombinant Ag panels
Field trials are expensive, not possible across many products, specific in time and population

<table>
<thead>
<tr>
<th>Study</th>
<th>TP</th>
<th>FP</th>
<th>FN</th>
<th>TN</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinkhumba 2010</td>
<td>481</td>
<td>464</td>
<td>26</td>
<td>346</td>
<td>0.95 [0.93, 0.97]</td>
<td>0.43 [0.39, 0.46]</td>
</tr>
<tr>
<td>Ishengoma 2011</td>
<td>3343</td>
<td>1695</td>
<td>436</td>
<td>12739</td>
<td>0.88 [0.87, 0.89]</td>
<td>0.88 [0.88, 0.89]</td>
</tr>
<tr>
<td>Lemma 2011a</td>
<td>402</td>
<td>114</td>
<td>51</td>
<td>1855</td>
<td>0.89 [0.85, 0.92]</td>
<td>0.94 [0.93, 0.95]</td>
</tr>
<tr>
<td>Lemma 2011b</td>
<td>377</td>
<td>97</td>
<td>76</td>
<td>1872</td>
<td>0.83 [0.79, 0.87]</td>
<td>0.95 [0.94, 0.96]</td>
</tr>
<tr>
<td>Mubi 2011</td>
<td>282</td>
<td>442</td>
<td>48</td>
<td>657</td>
<td>0.85 [0.81, 0.89]</td>
<td>0.60 [0.57, 0.63]</td>
</tr>
<tr>
<td>Premji 1994</td>
<td>213</td>
<td>24</td>
<td>40</td>
<td>103</td>
<td>0.84 [0.79, 0.88]</td>
<td>0.81 [0.73, 0.88]</td>
</tr>
<tr>
<td>Ratsimbaosa 2012</td>
<td>94</td>
<td>12</td>
<td>4</td>
<td>80</td>
<td>0.96 [0.90, 0.99]</td>
<td>0.87 [0.78, 0.93]</td>
</tr>
<tr>
<td>Tiono 2013</td>
<td>276</td>
<td>109</td>
<td>6</td>
<td>125</td>
<td>0.98 [0.95, 0.99]</td>
<td>0.53 [0.47, 0.60]</td>
</tr>
</tbody>
</table>

Figure 10 Forest plot of RDT performance when performed by CHWs (no subgroup analyses). Lemma 2011a = Paracheck Pf, Lemma 2011b = Parascreen pan/p.

200+ malaria RDT in the market; 60+ manufacturers)
International collaboration

- Between 2002-2008, WHO, TDR, FIND, US CDC and other partners developed methods, characterized (microscopy, PCR, ELISA), diluted and stored wild type *P. falciparum* and *P. vivax* clinical samples from Africa, South America and South East Asia.
WHO-FIND strategy for QA of RDT-based diagnosis

Supply chain management

Manufacture
- Product development
- Availability of common reference standards

Stage 1: Product testing
- Evaluate product performance

Transport and storage

Stage 2: Lot testing
- Confirm product quality on arrival in country before distribution to the field

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

Stage 3: QC at point of use (positive control wells)
- Ensure that RDTs have maintained accuracy through transport and storage

Before purchase
- Before distribution
- Before use
Current Product Testing

Comparative evaluation of commercially-available antigen-detecting malaria rapid diagnostic tests – RDTs.

Evidence of quality manufacturing

RDTs to specimen bank with temperature monitor

- Performance versus panel
- Stability
- Ease-of-Use assessment

Review of results by technical group
Results released to manufacturers

Longer-term stability test by manufacturer

Final publication

5 years

$ R6
Product Testing (at US CDC)

- **Performance** – panel detection score, false-positive and invalid rates
  - Phase 1 – 20 cultured *P. falciparum* samples; 2 lots; 1 RDT/lot @2000p/µl; 2 RDT/lot @ 200p/µl + 20 clean negative samples in R6
  - Phase 2
    - *P. falciparum* (100), *P. vivax* (35), 2 lots; 1 RDT/lot @2000p/µl; 2 RDT/lot @ 200p/µl
    - 1000 negative samples (mixed clean and other disease conditions)

- **Heat stability** (4°C, 35°C, 45°C; 75% humidity x 60 days)

- **Ease of use assessment**
  - blood safety, instructions quality, no. timed steps, RDT anomalies
### Malaria Antigen Targets for RDTs

#### Table 3. Antigen targets of rapid diagnostic tests for malaria

<table>
<thead>
<tr>
<th>Plasmodium species</th>
<th>HRP2</th>
<th>pLDH-Pf</th>
<th>pLDH-pan</th>
<th>pLDH-Pvom</th>
<th>pLDH-Pv</th>
<th>Aldolase</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. falciparum</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>P. vivax</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>P. malariae</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. ovale</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

HRP2 – histidine-rich protein 2  
pLDH – *Plasmodium* lactate dehydrogenase  
Pf – *P. falciparum*  
pan – all *Plasmodium* species  
Pvom – *P. vivax*, *ovale* and *malariae*  
Pv – *P. vivax*

Box 2: Performance measures in WHO product testing and in field settings: PDS versus clinical sensitivity

WHO Malaria RDT Product Testing
Primary performance measure: PDS indicates which products are likely to be more sensitive in the field, particularly in populations with low-density infections.

Malaria endemic setting
Performance measure: sensitivity is the proportion of the population studied who have malaria for whom the test is positive.

- high, moderate, low transmission
- immune, non-immune
- vulnerable groups

Reference panels: two fixed parasite densities allows discrimination in RDT performance.

Patients have varying parasite density. Most RDTs for *P. falciparum* and *P. vivax* perform well for a parasite density > 2000 parasites/µL, but clinically significant densities < 200 parasites/µL may be missed. The "overall" test performance will nevertheless be classified as very good in a field evaluation.
Antigen concentrations (HRP2, pLDH, aldolase) in panel samples Rounds 1-5

Box 4. Explanations for variable antigen concentrations in samples with the same parasite density
- variation in antigen expression among isolates
- different durations of infections (accumulating antigens)
- different parasite growth stages at the time of collection (expressing different levels of antigens)
- presence of circulating HRP2 from previous cycles of growth
- HRP2 produced by parasites sequestered in the host’s vascular tissues that cannot be accounted for in the estimate of parasite density on the blood slide (29)
Performance measure: Panel detection score

Box 1: Example calculation of **panel detection score** and **positivity rate** for product A against a sample density of 200 parasites/μL.

The first reading was at the minimum time specified by the manufacturer; the second reading was up to 30 min later. A sample is considered detected only if all first test readings, from both lots, are positive, i.e. readings a, b, c and d must be positive.

![](image)

*second reading results are for internal use only*

<table>
<thead>
<tr>
<th>P. falciparum sample</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>Sample NOT detected</th>
<th>Sample detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In this example, only one of three samples was positive all four times it was tested; the PDS is therefore 1/3 = 33%.

The **positivity rate** is calculated as the percentage of all tests of a particular product that returned a positive test result at the manufacturers’ recommended minimum reading time when tested against a *P. falciparum* or *P. vivax* sample.

In the above example, the positivity rate is: 9/12 = 75%.

The **positivity rate** is always greater than the PDS, except when the PDS and the positivity rate are both 100%.
Rounds 1-5

- Published Rounds 1-5
  - 206 RDTs evaluated (147 unique products)
- Round 5: 42 RDTs (23 resubmissions (10 compulsory)
  - 31 combo, 9 Pf, 2 pan (34 manufacturers)

- Round 6
  - 41 RDTs (30 combo, 11 Pf (22 manufacturers))
Results: PDS @ 200 and 2000 p/µL

Figure 10: Phase-2 P. falciparum panel detection score of malaria RDTs at low (200) and high (2000) parasitemia (parasites/µL) according to target antigen type (HRP2 or pLDH)²

Figure 11: Phase-2 P. vivax panel detection score of malaria RDTs at low (200) and high (2000) parasitemia (parasites/µL) according to target antigen type (aldolase, pLDH)³

² 4 (4%) of the 100 P. falciparum dilution samples had 200 and 5000 parasites/µL, and 2 (6%) of the 35 P. vivax dilution sample sets had 200 and 5000 parasites/µL.

³ Phase-2 evaluation panel consisted of 100 clinical blood samples containing wild-type P. falciparum. RDTs performed = 2 tests x 2 lots at 200 p/µL and 1 test x 2 lots at 2000 p/µL.

A sample is considered detected only if all RDTs from both lots read by the first technician, at the minimum specified reading time, are positive.
Figure S3: Panel detection score of malaria combination RDTs, meeting WHO procurement criteria for false-positive and invalid rates, in phase 2 of rounds 2–5 against wild-type (clinical) samples containing *P. falciparum* and *P. vivax* at low (200) parasite density (parasites/μL).

Panel detection score - A sample is considered detected only if all RDTs from both lots read by the first technician, at the minimum specified reading time, are positive.
PT results are the basis for WHO procurement criteria

Box 3: WHO selection criteria for the procurement of RDTs

Products should be selected in line with the following set of criteria, based on the results of the assessment of the WHO Malaria RDT Product Testing Programme:

(A) For the detection of *Plasmodium falciparum* (Pf) in all transmission settings the panel detection score (PDS) against Pf samples should be at least 75% at 200 parasites/µL.

(B) For the detection of *Plasmodium vivax* (Pv) in all transmission settings the panel detection score (PDS) against Pv samples should be at least 75% at 200 parasites/µL.

(C) The false positive rate should be less than 10%.

(D) The invalid rate should be less than 5%.

Only products meeting performance criteria outlined in A, B, C and D are recommended for procurement

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**Eligible for tender: 58 RDTs (24 Pf, 31 combo, 2 pan; 1 Pv only)**

**Further considerations:**

- Stability
- Ease of use and training requirements
- Price
- Lot testing
PDS\textsubscript{Pf} and PDS\textsubscript{PV} were significantly lower in compulsory resubmissions as compared to voluntary resubmissions.

### Table 1b: Products due for compulsory resubmission in round 5

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Product name</th>
<th>Catalogue number</th>
<th>Participation in round 5\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Access Bio, Inc.</td>
<td>CareStart™ Malaria pLDH (PAN)</td>
<td>G0111</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>CareStart™ Malaria HRP2/pLDH (Pf/PAN) COMBO</td>
<td>G0131</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>CareStart™ Malaria HRP2 (Pf)</td>
<td>G0141</td>
<td>Yes</td>
</tr>
<tr>
<td>Acon Laboratories, Inc</td>
<td>Malaria Plasmodium falciparum Rapid Test Device (Whole Blood)</td>
<td>IMA-402</td>
<td>No</td>
</tr>
<tr>
<td>Amgenix International, Inc.</td>
<td>OnSight - ParaQuick (Pan, Pf) Test</td>
<td>536-25DB</td>
<td>No</td>
</tr>
<tr>
<td>Biosynex</td>
<td>Immuquick Malaria Falciparum</td>
<td>0502_K25</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Immuquick Malaria +4</td>
<td>0506_K25</td>
<td>No</td>
</tr>
<tr>
<td>Diagnostics Automation/Cortez Diagnostics Inc.</td>
<td>Malaria Pf/Vivax</td>
<td>172110P-25</td>
<td>No</td>
</tr>
<tr>
<td>Human GmbH</td>
<td>Hexagon Malaria</td>
<td>58051</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Hexagon Malaria Combi</td>
<td>58024</td>
<td>No</td>
</tr>
<tr>
<td>IND Diagnostic Inc.</td>
<td>One Step Malaria Antigen Strip</td>
<td>820-1</td>
<td>No</td>
</tr>
<tr>
<td>Innovatek Medical Inc.</td>
<td>Quickstick Malaria Antigen Test\textsuperscript{b}</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Intec Products, Inc.</td>
<td>ADVANCED QUALITY TM MALARIA (p.f) POCT</td>
<td>ITP11002TC1</td>
<td>Yes</td>
</tr>
<tr>
<td>Inverness Medical Innovations, Inc.</td>
<td>Binax Now Malaria</td>
<td>IN660050</td>
<td>No</td>
</tr>
<tr>
<td>J. Mitra &amp; Co. Pvt. Ltd</td>
<td>Advantage P.f. Malaria Card</td>
<td>IR016025</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Advantage Pan Malaria Card</td>
<td>IR013025</td>
<td>Yes</td>
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<td>Advantage Mal Card</td>
<td>IR212025</td>
<td>Yes</td>
</tr>
<tr>
<td>Premier Medical Corporation Ltd.</td>
<td>First Response\textsuperscript{®} Malaria Ag HRP2</td>
<td>I13FRC</td>
<td>Yes</td>
</tr>
<tr>
<td>Span Diagnostics</td>
<td>Parahit-Total Device Rapid Test for \textit{P. falciparum} and \textit{Pan malaria} species</td>
<td>25989</td>
<td>No</td>
</tr>
<tr>
<td>Standard Diagnostics</td>
<td>SD Bioline Malaria Ag Pf</td>
<td>05FK50</td>
<td>Yes</td>
</tr>
<tr>
<td>Unimed International</td>
<td>FirstSign – Malaria PF Card Test</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>FirstSign – ParaView-2 (Pv + Pf) Card Test</td>
<td>2102CB-25</td>
<td>No</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The results of the first tests of the products in this list that were not retested in round 5 have been removed from tables S2 and S3 and figs S1 and S2.

\textsuperscript{b} Co-listed with IND Diagnostics - One Step Malaria Antigen Strip (820-1)
**RDT anomalies**

### a) Observations on the test strip
- **Red background**
  - Background staining is relatively common. In this example, the result is positive as test lines are positive; however, a more intense red background may obscure weak positive test lines, giving false-negative results.

- **Incomplete clearing**
  - Poor clearing of blood may obscure weak positive test lines, giving false-negative results.
  - In this example, the result is positive as the test line is visible.

### b) Observations of flow problems
- **Failure to flow**
  - Blood and buffer clog the strip.

- **Irregular migration that obscures test line(s)**
  - One portion of the test band was not dry during wicking, obscuring the observation of blood/buffer that may obscure test lines.

- **Irregular migration**
  - One portion of the test band was not dry during wicking, obscuring the test line.
  - In this example, the test line is clearly visible.

### c) Observations on test lines
- **Ghost test line(s)**
  - White line on a strip example, the result line is not dark and visible.

- **Patchy broken test line(s)**
  - The test line is visible but irregular (broken).

- **Diffuse test line(s)**
  - Test line wider than control, without clearly defined edge.

### d) RDT structural problems
- **Strip misplaced in the cassette**
  - Strip can be seen only partially in the results window.

- **Specimen pad not seen in sample window**
  - Normally, the colour of the conjugated antibody can be seen in the sample window (commonly purple, pink or blue).

### Table 8: Observations on RDT production lots that might affect interpretation of the results

<table>
<thead>
<tr>
<th>Observations/anomalies</th>
<th>No. (%) of products with at least one recorded observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red background</td>
<td>42 (100)</td>
</tr>
<tr>
<td>Incomplete clearing</td>
<td>42 (100)</td>
</tr>
<tr>
<td>Failure to flow</td>
<td>26 (61.9)</td>
</tr>
<tr>
<td>Shift or misplacement of strip</td>
<td>10 (23.8)</td>
</tr>
<tr>
<td>Ghost lines</td>
<td>7 (16.7)</td>
</tr>
<tr>
<td>Diffuse test lines</td>
<td>2 (4.8)</td>
</tr>
<tr>
<td>Patchy broken test line</td>
<td>2 (4.8)</td>
</tr>
</tbody>
</table>
WHO has started in 2007 the prequalification of malaria RDTs according to the following procedure:

So far the following RDTs has been prequalified:

- SD BIOLINE Malaria Ag P.f (05FK50/05FK53)
- SD BIOLINE Malaria Ag P.f/Pan (05FK63 and 05FK60)

WHO PQ Lab evaluation = WHO Malaria RDT Product Testing
Panel Detection Score (PDS) of Malaria RDT submitted for lot-testing (for pre/post procurement)

<table>
<thead>
<tr>
<th>Year</th>
<th>PDS* ≥75% (high performance criteria)</th>
<th>PDS* ≥50% (WHO medium performance criteria)</th>
<th>PDS* &lt;50% including False Positive (outside WHO criteria)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>71.19%</td>
<td>1.44%</td>
<td>28.81%</td>
</tr>
<tr>
<td>2008</td>
<td>88.49%</td>
<td>1.44%</td>
<td>10.07%</td>
</tr>
<tr>
<td>2009 Rd 1 Apr.</td>
<td>93.42%</td>
<td>0.00%</td>
<td>6.58%</td>
</tr>
<tr>
<td>2010 Rd 2 May</td>
<td>95.76%</td>
<td>0.00%</td>
<td>4.24%</td>
</tr>
<tr>
<td>2011 Rd 3 Dec</td>
<td>89.04%</td>
<td>0.35%</td>
<td>6.58%</td>
</tr>
<tr>
<td>2012 Rd 4 Dec</td>
<td>94.18%</td>
<td></td>
<td>5.11%</td>
</tr>
<tr>
<td>Jan-Jun 2013</td>
<td>99.04%</td>
<td></td>
<td>0.38%</td>
</tr>
</tbody>
</table>

Not submitted to Product Testing: 4.38% 0.35% 0.58%
Based on Global Fund and PMI data (compiled by CHAI and UNITAID)

- Three manufacturers won 90% of tenders in 2012
- Four won 98% in 2013
- 90% of public sector supplies depends on 2 manufacturers
Impact on manufacturers

• Following Round 5

  – One prequalified product – Immunoquick Malaria falciparum (0502_K 25,50,100 Dipstick) (Biosynex) is delisted (PF PDS <75% and ++ red background)

  – One market leader combination test, First Response pLDH-HRP2 Combo Test, I16FRC (Premier Medical Corporation) scored P.vivax PDS 74.3% (Pf PDS 85%).

  • Comparable to scores in Rounds 1 and 2 – PDS 75%.

    – 2014 NOT eligible for WHO tender or procurement

    – Procured by Ethiopia, Tanzania, DRC, Madagascar, Rwanda, India, Pakistan, Myanmar, Cambodia, Indonesia
Limitations of current system

- Need to reduce costs ++ to ensure sustainability and reasonable manufacturer payments

- Need to standardize panels across time and space

- Need to make panels available to manufacturers (same as are used for product testing and lot-testing)

- Need to provide countries with standard, reliable, acceptable materials for lot-testing (there will be increased requirement for in-country testing of RDTs in the future)
Recombinant antigen based system

Identification of candidate antigens:
- Acquisition of recombinant antigens from other institutions
- Procurement of commercially available recombinant antigens
- Synthesis and expression of new recombinant antigens

Quality evaluation:
- Calculation of protein concentration by absorbance
- Evaluating purity by polyacrilamide gels
- Assessing immunodetection and concentration curves by ELISA
- Testing storage and temperature stability

Performance evaluation:
- Equivalence testing by RDTs to compare PDS (Panel Detection Score) between selected recombinant antigens and parasites
# 2013-2017 plan funded by UNITAID

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Step 1:</strong></td>
<td><strong>Start</strong></td>
<td><strong>Step 2:</strong></td>
<td><strong>Step 3:</strong></td>
<td><strong>Step 4:</strong></td>
</tr>
<tr>
<td></td>
<td>Establish patient-derived sample panels</td>
<td>Develop and evaluate recombinant antigen panels</td>
<td>Scale-up and launch recombinant antigen panels</td>
<td>Manufacture and distribute reference materials</td>
</tr>
<tr>
<td></td>
<td>Establish lot testing process</td>
<td>Ongoing lot testing based on cultured parasites</td>
<td>Roll-out lot testing based on recombinant antigens</td>
<td>Local lot testing financed by purchaser</td>
</tr>
<tr>
<td></td>
<td>Product testing round 1 to 3</td>
<td>Ongoing product testing round 4 &amp; 5</td>
<td>Product testing based recombinant panel and partly financed by IVD suppliers</td>
<td>Product testing financed by IVD suppliers</td>
</tr>
</tbody>
</table>

**Cost:**
- 2003 -2011: $$$$$
- 2011-2014: $$$$$$
- 2015-2016: $$$
- 2017: $
Thank you!

- FIND
- US CDC
- Hospital for Tropical Disease, UK
- Queensland Institute of Medical Research, Australia
- Army Malaria Institute, Australia
- Research Institute Tropical Medicine, The Philippines
- Institute Pasteur Cambodia
- Collection sites: CIDEIM (Colombia), DMR (Myanmar), KEMRI (Kenya), EHNRI (Ethiopia), IHRDC (Tanzania), IMT (Peru), IPB (Central Africa Republic), IPM (Madagascar), UCAD (Senegal), UL (Nigeria)