Fast Rapid Diagnostic tests for diagnosis of malaria.

Tom van Gool
Academic Medical Centre, Amsterdam
The importance of malaria diagnosis

Without vaccines being available the major strategy to combat malaria is currently prompt diagnosis, treatment and prevention.
Fast diagnosis: low parasitaemia, low risk for complications!!
New(er) tests for malaria diagnosis

- Quantitative Buffy Coat (QBC)
- Automatic analysers (CD4000)
- Polymerase Chain Reaction (PCR)
- Antigen detection (RDT)
Antigen-detection for malaria

- Fast method (5-15 min)
- Easy to use, no specific expertise needed
- No specific apparatus needed
Antigens used in current tests:

1) HRPII (Histidine Rich Protein II, *P. falciparum*)

2) Aldolase (present in all species)

3) Parasitic Lactate Dehydrogenase (pLDH)
   - a) pLDH specific for *P. falciparum*
   - b) pLDH specific for *P. vivax*
   - c) pan-pLDH: present in all species
ICT NOW® Malaria and ICT Combo Cassette

Positive control

P.falciparum HRP II

Aldolase

Combo cassette
OptiMal-IT

Positive control

Pan malaria LDH

P. falciparum LDH
Palutop+4® and Core Malaria

Positive control

Pan malaria LDH

P. vivax LDH

P. falciparum HRP II
Overview some commercial antigen tests

Antigen test

**Antigens**

- *Malaria species*
  - HRP2
  - *P. falciparum*
  - Aldolase
    - *All species*
    - HRP2
    - *P. falciparum*
    - Pf LDH
      - *P. falciparum*
      - Pan LDH
        - *All species*
        - HRP2
        - *P. falciparum*
        - Pv LDH
          - *P. vivax*
          - Pan LDH

**Core**

**Optimal 48/ IT**

**ICT**

**Combo cassette**

**Palutop**

Cheap!

Technical identical
Experience with antigen tests (RDT) from literature

Rapid, fast and reliable

Sensitivity from 80-100%

Specificity from 90-100%

So............
Is, with the new tests, classical microscopy outdated?

Remote areas in tropics …..?

Places in tropics with some basic infrastructure….? 

Laboratories in tropics with good infrastructure…..?

Laboratories in western countries at daytime…….?

Laboratories in western countries at night shifts…..?
Study towards use of dipsticks in routine clinical practice in the Netherlands and Surinam
Study sites

Bureau voor Openbare Gezondheidszorg
Laboratory for Parasitology
Paramaribo, Suriname

Laboratory for Parasitology,
Harbour Hospital, Rotterdam and
Academic Medical Centre,
Amsterdam
Microscopic methods used studied (I):

- Thick smear: Giemsa and Fields stained (1000 x)
- Thin smear (Diff Quick) 200 fields (1000 x), 5 min.
- QBC
ICT-NOW
Netherlands

Optimal 48/IT
Netherlands

Combo
Surinam

Core
Surinam

HRPII and aldolase
pLDH Pf
pLDH Ps

HRPII and aldolase
pLDH Pf
pLDH Pv
pLDH Pan
Patients included in the study

Gold standard: 800 fields 1000x Giemsa

<table>
<thead>
<tr>
<th>Study site</th>
<th>Patients included in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no. cases</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 1 Netherlands</td>
<td>383</td>
</tr>
<tr>
<td>Study 2 Surinam</td>
<td>331</td>
</tr>
</tbody>
</table>
Sensitivity and specificity
antigen tests
Sensitivity of antigen tests

**Netherlands**

<table>
<thead>
<tr>
<th>Species</th>
<th>ICT</th>
<th>Combo</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. falciparum</td>
<td>82 (98.8%)</td>
<td>72 (98.7%)</td>
</tr>
<tr>
<td>P. vivax</td>
<td>15 (75.0%)</td>
<td>47 (95.9%)</td>
</tr>
<tr>
<td>P. ovale</td>
<td>1 (20.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>P. malariae</td>
<td>1 (100%)</td>
<td>6 (75.0%)</td>
</tr>
</tbody>
</table>

- ICT sensitivity over all: 91%
- Combo sensitivity over all: 96%

**Surinam**

<table>
<thead>
<tr>
<th>Species</th>
<th>ICT</th>
<th>Combo</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. falciparum</td>
<td>74 (98.7%)</td>
<td>66 (88.0%)</td>
</tr>
<tr>
<td>P. vivax</td>
<td>47 (95.9%)</td>
<td>45 (91.8%)</td>
</tr>
<tr>
<td>P. malariae</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

- ICT sensitivity over all: 94%
- Combo sensitivity over all: 95%

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Specificity of antigen tests

<table>
<thead>
<tr>
<th>Antigen test</th>
<th>Country</th>
<th>No. cases</th>
<th>No. positive for sexual stages of malaria</th>
<th>No. patients with false positive band (Specificity %)</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>120/122</td>
<td>120/122 (98,4)</td>
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<tr>
<td></td>
<td></td>
<td>266/272</td>
<td>266/272 (97,8)</td>
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<tr>
<td></td>
<td></td>
<td>121/122</td>
<td>121/122 (99,1)</td>
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<tr>
<td></td>
<td></td>
<td>268/272</td>
<td>268/272 (98,5)</td>
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<td></td>
<td></td>
<td>12/12</td>
<td>12/12 (100)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>269/272</td>
<td>269/272 (98,9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Netherlands</td>
<td>Surinam</td>
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<tr>
<td>Sensitivity P. falciparum (%)</td>
<td>98,8</td>
<td>88,2</td>
<td></td>
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</tr>
<tr>
<td>Sensitivity all species (%)</td>
<td>90,8</td>
<td>83,7</td>
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<tr>
<td>Specificity (%)</td>
<td>97,8</td>
<td>100</td>
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<tr>
<td>Cost of test €</td>
<td>9,76</td>
<td>3,16</td>
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</table>

* only Dutch study used
Combination of antigen test and microscopy: the best of two worlds?
Conclusions antigen tests

- Antigen tests easy to use, fast results
- Good sensitivity (less than QBC and Thick Smear)
- HRPII better for *P.falciparum* as specific pLDH test
- Aldolase and pLDH good results with other species
- In general good specificity
- Can occasionally be false negative with high(er) parasitaemia
- Can occasionally be false positive
Conclusions antigen test and thin smear

- Combination of antigen tests and thin smear highly efficient: sensitivities: 97%- 99%

- With this combination no major mistakes can be made and parasitemia can reliably be calculated.

- *Combination easy to perform, also in less experienced hands!*
Can with an antigen test the parasitemia of *P. falciparum* be established?
Relation with aldolase band and parasitemia?

Study among 123 patients with *P. falciparum* with known parasitemia
Use of aldolase band for estimation parasitaemia *P. falciparum* infection

Only HRPII (T1)?
(often in imported malaria)

→ *P. falciparum* infection, parasitemia always lower as 1%.

HRPII and aldolase positive?

→ Parasitaemia *P. falciparum* most likely higher as 1% but lower parasitaemia also possible
Is with the new tests classical microscopy outdated?

Remote areas in tropics ……yes.
Places in tropics with some basic infrastructure…? Laboratories in tropics with good infrastructure….. no Laboratories in western countries at daytime…… no Laboratories in western countries at night shifts:

antigen test and thin smear!