Malaria Rapid Diagnostic Tests: role and place in the diagnosis of malaria

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Malaria: an overview

1. Plasmodium falciparum
2. Plasmodium vivax
3. Plasmodium ovale
4. Plasmodium malariae
5. Plasmodium knowlesi

Most serious

Travellers

- Risk = 0.15 - 0.25% of travellers
- 80% develop symptoms only on return home
- Case fatality rate 0.6 – 3.8%, depends on diagnostic delay
- 50% of smears
- 60% of diagnosis outside office hours
- P. falciparum 60% - 90%

World-wide

- 40% of world population
- 2,700,000 deaths/year
- 90% Africa, < 5 years
- Semi-immunity in > 5 years
- Epidemics: all ages

- Trophozoites
- Schizonts
- Gametocytes

Figure 1: Malaria global map
Malaria diagnosis: recommendations

- Parasite-based diagnosis essential
  - Malaria Yes or No
  - Species
  - Parasitaemia
  - Stages/Pigment

Post treatment follow-up

Malaria diagnosis, before referral

1. Malaria Yes or No
2. P. falciparum versus non-falciparum
3. Parasitaemia
   - > 2% of red blood cells infected = alert

Staff
  - (training & expertise)
  - Off-hours

![Graph showing malaria cases per laboratorium per jaar in België from 1984 to 2004.]

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases per Laboratorium</th>
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<tbody>
<tr>
<td>1984</td>
<td>0</td>
</tr>
<tr>
<td>1986</td>
<td>0.5</td>
</tr>
<tr>
<td>1988</td>
<td>1</td>
</tr>
<tr>
<td>1990</td>
<td>1.5</td>
</tr>
<tr>
<td>1992</td>
<td>2</td>
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<tr>
<td>1994</td>
<td>2.5</td>
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<td>1996</td>
<td>3</td>
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<td>1998</td>
<td>3.5</td>
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<tr>
<td>2000</td>
<td>4</td>
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<td>2002</td>
<td>4.5</td>
</tr>
<tr>
<td>2004</td>
<td>5</td>
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![Thick film and Thin film images showing parasite detection and quantification.

- Thick film:
  - Parasite detection.
  - Quantification [parasitaemia /µl of blood].

- Thin film:
  - Differentiation between species.
  - Quantification [% infected RBC].

50,000/µl = 1% of Red Blood Cells
100/µl = 0.002% of RBC

![Graph showing quantification of parasites per µl of blood with examples.]

2
Antigens targeted by malaria RDTs

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Targets</th>
<th>Persistence</th>
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</thead>
<tbody>
<tr>
<td>HRP-2</td>
<td>1. P. falciparum</td>
<td>Viable trophozoites and gametocytes</td>
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<tr>
<td></td>
<td>2. All species (pan)</td>
<td></td>
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<tr>
<td></td>
<td>3. P. vivax</td>
<td>Persistence up to 43 days after treatment</td>
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<tr>
<td>pLDH</td>
<td>1. P. falciparum</td>
<td>Viable trophozoites and gametocytes</td>
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<td>No persistence</td>
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<td>3. P. vivax</td>
<td>Follow-up of treatment</td>
</tr>
<tr>
<td>Aldolase</td>
<td>All species</td>
<td>No persistence</td>
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Place for Malaria rapid diagnostic tests (RDT)?
Malaria Rapid Diagnostic Tests: formats

- Dipstick
- Card
- Plastic cassette
- Hybrid cassette-dipsticks

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<tr>
<td>P. falciparum-spec.</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Pan-specific</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>P. vivax-specific</td>
<td>+</td>
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P. falciparum
First generation
Cheap
Two-band

P. vivax
Two-band

P. falciparum/mixed versus non-falciparum
Three-band
**Malaria Rapid Diagnostic Tests: principle**

“Lateral flow Immunochromatographic tests”

1. **Principle**
2. **Limitations**
3. **Place of malaria rapid diagnostic tests**
   - How to deal with these limitations?

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<td><strong>Three-band</strong></td>
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<td><strong>Four-band</strong></td>
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Ab1 = anti-HRP2 (mouse) + gold (conjugated) = capture Ab
Ab2 = anti-HRP2 (other epitope) (mouse) = detecting Ab
Ab3 = anti-mouse Ig (goat) = control Ab
Immunoassay: Limitations

1. Sensitivity: Detection limit and Prozone effect
2. Specificity: False-positive reactions
3. No (semi)quantification
4. Faint to strong lines
5. Species identification
6. Delayed reading (Backflow)

1. Sensitivity  
   Detection Limit

   *P. falciparum* malaria: sensitivity 88 – 99%
   High sensitivity above 100 parasites/µl
   HRP-2 performs slightly better than pLDH
   Possibility of HRP-2 mutations/deletions

   *P. vivax* malaria: sensitivity 70%
   increasing to 95% at parasitaemia > 500/µl

   Poor sensitivity for *P. ovale/P. malariae*
   Most RDTs do NOT reliably detect
   *P. ovale* and *P. malariae*
Prozone effect or High Dose Hook Effect

False-negative results at high parasite densities

PATIENT FROM NIGERIA

Microscopy:
- *P. falciparum*
  - Parasitaemia: 30%

RDT:
- *Plasmodium non falciparum*

Prozone-effect in malaria rapid tests

False-negative/low reactions due to Antigen-excess
Only a single case report published

Prozone in hyperparasitemia:
- Faint instead of strong lines, occasionally negative result
- All but one HRP-2 tests affected, not found in LDH tests
- Volume of blood must be respected
- Dilution in NaCl/RDT kit’s diluent
2. False-positive reactions: Specificity

1. Rheumatoid factor
   Other infections (Schistosoma, hepatitis...) = rare

2. Persistence of HRP-2 after (self)-treatment
   Explains for a number of (seemingly) false-positives

3. Diluent replacement

Traveller, sub-Saharan Africa, fever:
Probability of P.falciparum before testing = 20%

Probability of P.falciparum malaria =
- after negative HRP2 test:  1.1% (0.6 - 1.9%)
- after positive HRP2 test:  97% (92 - 99%)

Exclusion power is not high enough to rely on Malaria RDT as the only diagnostic test for ruling out P. falciparum malaria

3. No reliable (semi)quantification

1. Line intensity related to parasitemia but considerable overlap

2. Presence of unique HRP-2 line in case of P.falciparum = parasitemia below 1,000/µl for some RDTs

4. Faint to strong test lines

Any visible line is a positive line (when read within recommended reading time)
Disregarding faint lines as negative is common error in tropical as well as in non-endemic settings
5. Species Identification

Two-bands: *P. falciparum* or *P. vivax* specific targets!
Possible cross-reactions of *P. falciparum* at high parasitaemia

Three-bands and four bands:
- *P. falciparum*: if visible line only with the *P. falciparum* specific target (HRP-2, Pf-pLDH)
- “*P. falciparum* or mixed infection” in other cases

6. Delayed Reading (Backflow)

1. Antigen-antibody interactions = time dependent:
   Delayed reading (beyond the recommended reading time) may increase the numbers of positives
   BUT
2. The so-called Backflow-phenomenon will cause non-specific (false-positive) readings
Place of RDTs in diagnosis malaria?

- Malaria Yes or No: Of considerable help
- P.falciparum versus non-falciparum: Of help
- Parasitemia: Of no help
- Point of Care: No place outside the laboratory (? travellers?)
- ALWAYS in conjunction with microscopy

How to deal with the Limitations?

1. Detection Limit: Repeat after 8h
2. Faint test lines: Any line is a positive line
3. Prozone: Dilute the sample (Respect the volume) (Have pLDH-test at hand)
4. Backflow: Do not read beyond the recommended reading time
What can you expect from the Institute of Tropical Medicine’s reference lab?

1. Reference
   - Confirmation (including Exclusion)
   - Advice on diagnosis


What can you expect from the Institute of Tropical Medicine’s reference lab?

1. Reference
   - Confirmation (including Exclusion)
   - Advice on diagnosis
2. WIV/ISP: External Quality Assessment
3. Evaluation of diagnostic kits

Thanks to ITM-team
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