

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

Jan Jacobs

Institute of Tropical Medicine
Antwerp



Malaria : an overview

1 *Plasmodium falciparum*

2 *Plasmodium vivax*

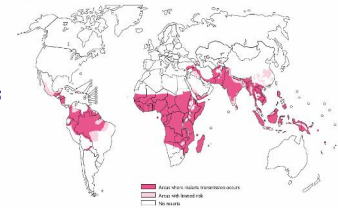
3 *Plasmodium ovale*

4 *Plasmodium malariae*

5 *Plasmodium knowlesi*

Most serious

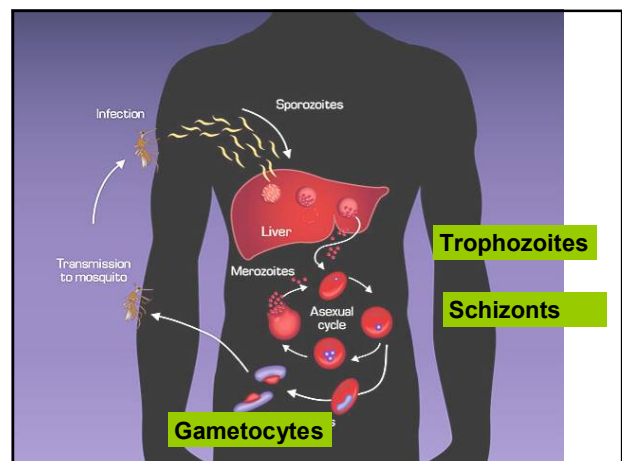
Figure 1: Malaria's global grip



World-wide
40% of world population
2.700.000 deaths/year
90% Africa, < 5 years
semi-immunity in > 5 years
epidemics: all ages

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%



Malaria diagnosis: recommendations

Parasite-based diagnosis essential

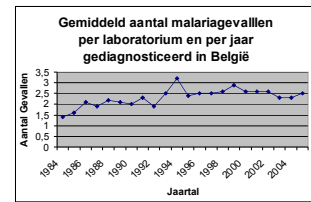
- Malaria Yes or No
- Species
- Parasitaemia
- Stages/Pigment

Post treatment follow-up

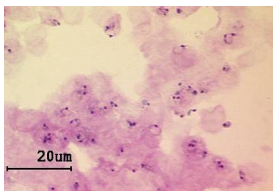
Malaria diagnosis, before referral

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



Thick film



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

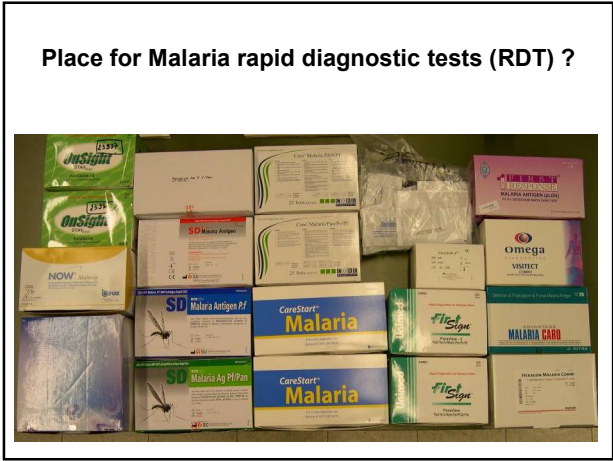
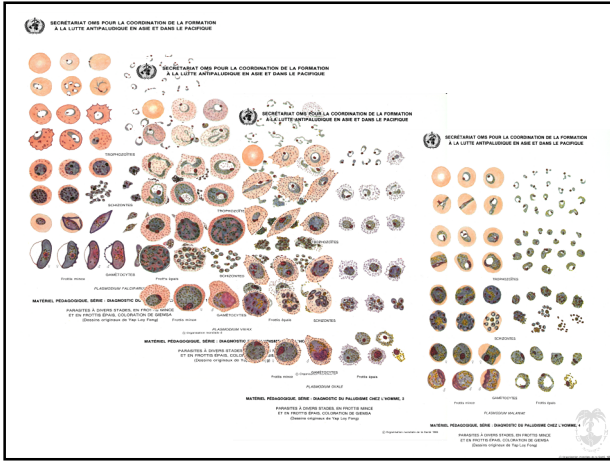
Thin film



- Differentiation between species.
- Quantification [% infected RBC].



50.000/µl = 1% of Red Blood Cells
100/µl = 0.002% of RBC



Place for Malaria rapid diagnostic tests (RDT) ?

Antigens targeted by malaria RDTs

HRP-2 Histidine-rich protein-2	P. falciparum	Trophozoites + young gametocytes Persistence up to 43 days after treatment
pLDH Parasite Lactate Dehydrogenase	1. P.falciparum 2. All species (pan) 3. P.vivax	Viable trophozoites and gametocytes No persistence Follow-up of treatment
Aldolase	All species	No persistence

Malaria Rapid Diagnostic Tests: Targets

	HRP-2	pLDH	Aldolase
P.falciparum- specific	+	+	
Pan-specific		+	+
P.vivax-specific		+	

Persistence

Viabile parasites

Malaria Rapid Diagnostic Tests: formats

Dipstick

Card

Plastic cassette

Hybrid cassette-dipsticks



	HRP-2	pLDH	Aldolase
<i>P.falciparum</i> -spec.	+	+	
Pan-specific		+	+
<i>P.vivax</i> -specific		+	

P.falciparum

First generation

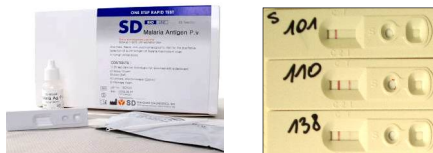
Cheap

Two-band



	HRP-2	pLDH	Aldolase
<i>P.falciparum</i> -spec.			
Pan-specific			
<i>P.vivax</i> -specific		+	

P. vivax
Two-band



	HRP-2	pLDH	Aldolase
<i>P.falciparum</i> -spec.	+	+	
Pan-specific		+	+
<i>P.vivax</i> -specific		+	


P.falciparum/mixed
versus
non-*falciparum*

Three-band




	HRP-2	pLDH	Aldolase
<i>P.falciparum</i> -spec.	+	+	
Pan-specific		+	+
<i>P.vivax</i> -specific		+	

P.falciparum*/mixed versus non-*falciparum
Three-band




	HRP-2	pLDH	Aldolase
<i>P.falciparum</i> -spec.	+	+	
Pan-specific		+	+
<i>P.vivax</i> -specific		+	

P.falciparum*/mixed versus non-*falciparum
Three-band



	HRP-2	pLDH	Aldolase
<i>P.falciparum</i> -spec.	+	+	
Pan-specific		+	+
<i>P.vivax</i> -specific		+	

P.falciparum*/mixed versus *P. vivax*/mixed versus other *Plasmodium
Four-band

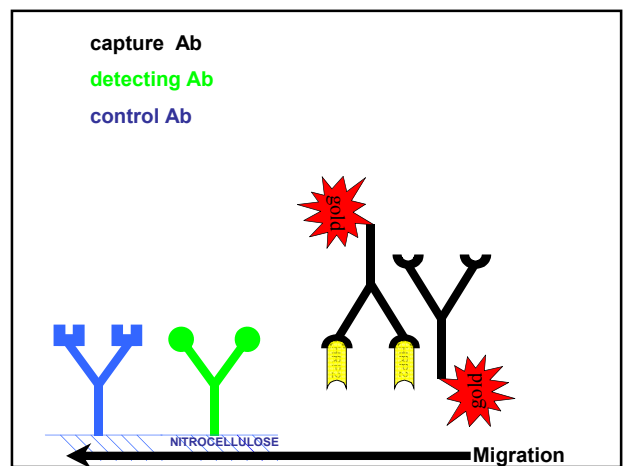
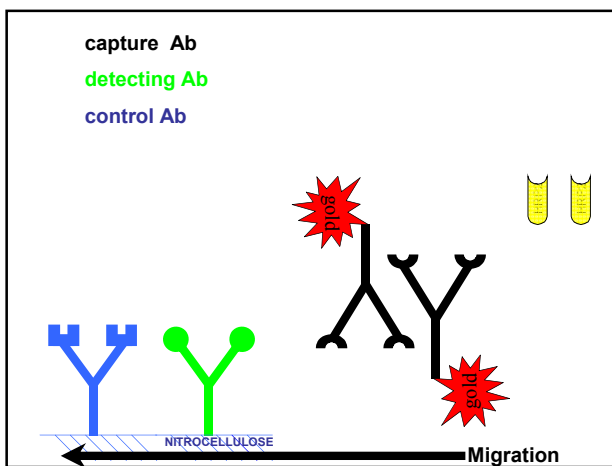
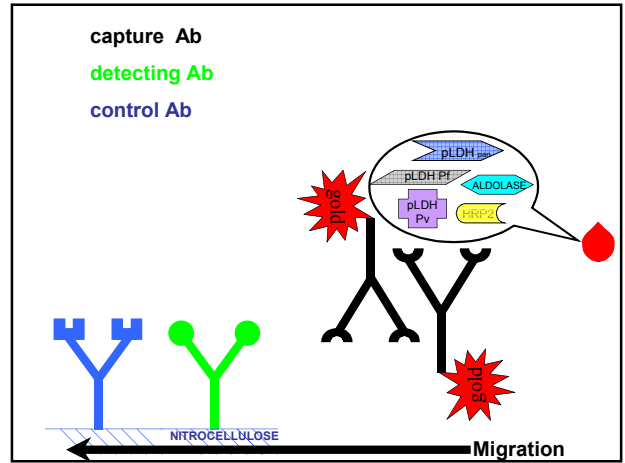
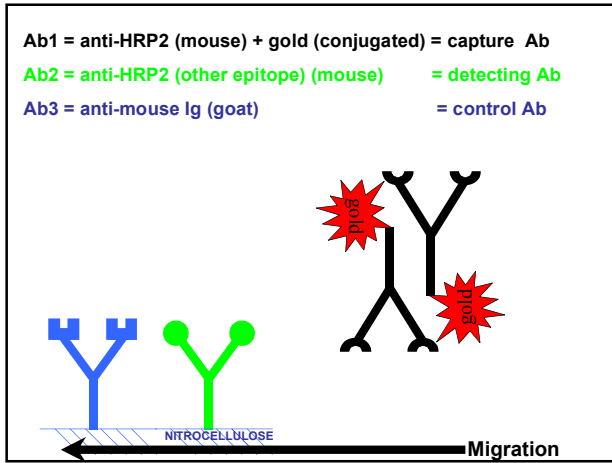


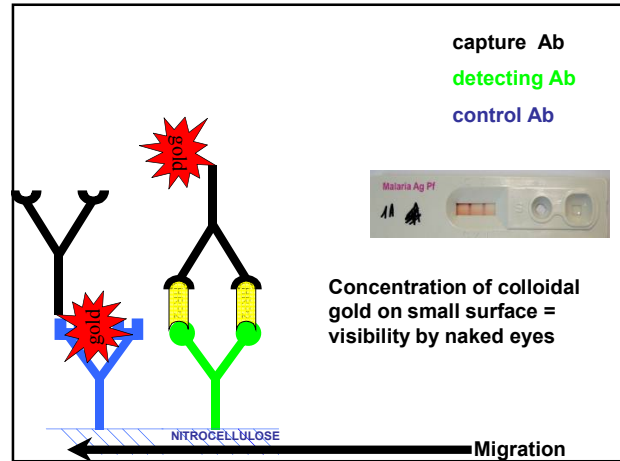
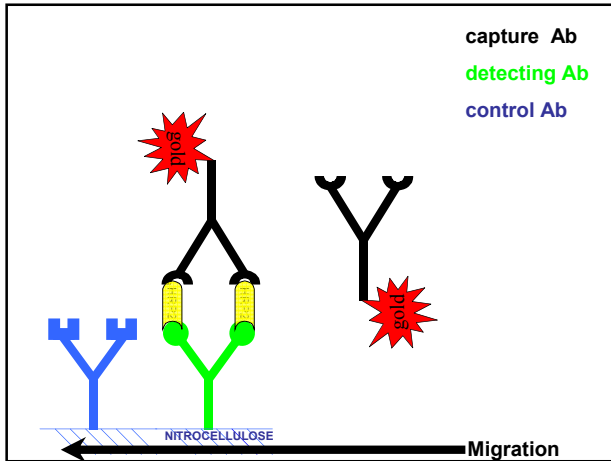
Malaria Rapid Diagnostic Tests: principle

“Lateral flow Immunochromatographic tests”

1. Principle
2. Limitations
3. Place of malaria rapid diagnostic tests

How to deal with these limitations?





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)

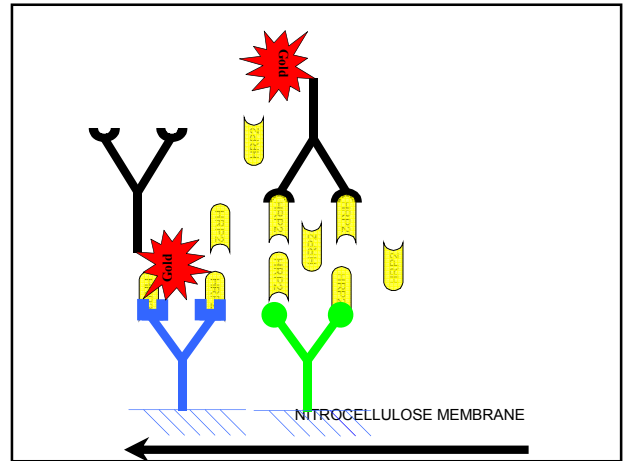
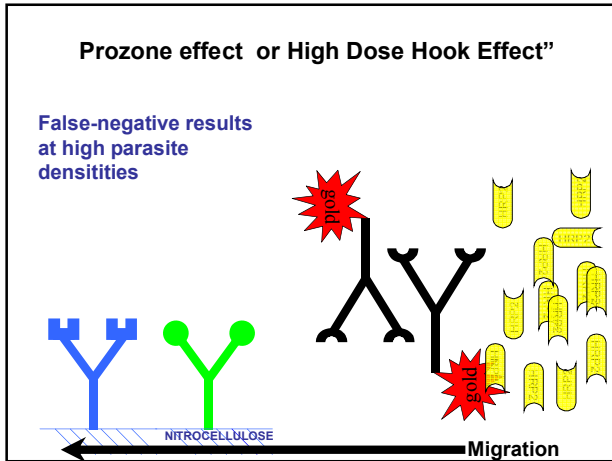


1. Sensitivity Detection Limit

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

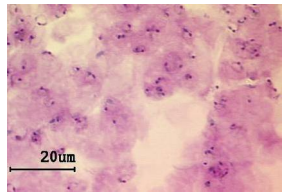
Poor sensitivity for *P. ovale*/*P. malariae*
Most RDTs do NOT reliably detect
P. ovale and *P. malariae*



PATIENT FROM NIGERIA


Microscopy:

- *P. falciparum*
- Parasitaemia : 30 %



RDT:

- *Plasmodium non falciparum*

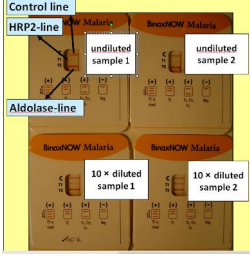


Prozone-effect in malaria rapid tests

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**

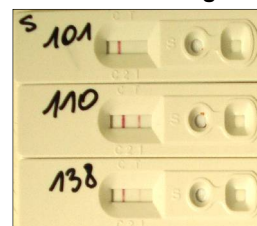
3. No reliable (semi)quantification

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/ μ l for some RDTs



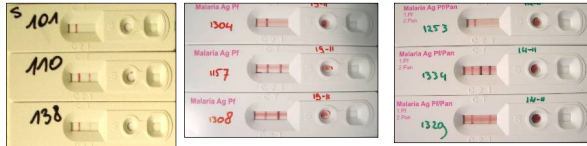
4. Faint to strong test lines



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

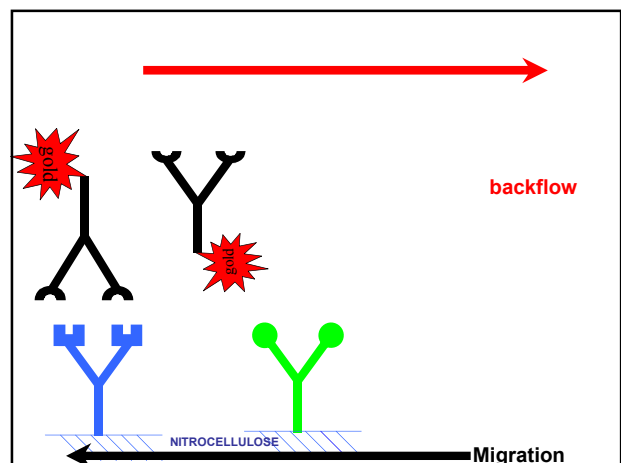
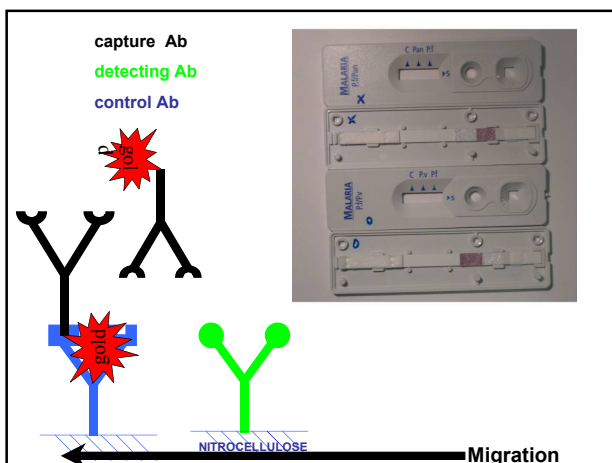
6. Delayed Reading (Backflow)

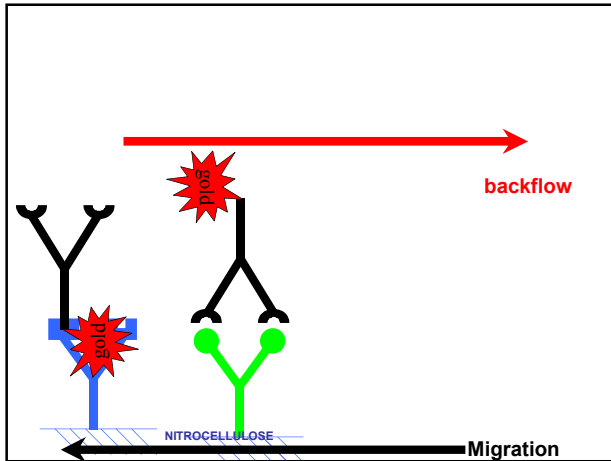
1. Antigen-antibody interactions = time dependent:

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





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

SO

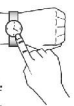
Respect the recommended reading time

(no "checks" afterwards!)

Interpret test results in 20-30 minutes.

20-30 mins

Caution:
Don't read test results after 30 minutes.
Reading too late can give false results.



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

https://www.iph.fgov.be/epidemie/epin/plabnl/N_Plasmodium.pdf

https://www.iph.fgov.be/epidemie/epin/plabnl/N_Plasmodium.pdf

TOE TOE STUREN:

- ongekleurde dikke druppel
- 2 ongekleurde bloedstrijkjes
- 2,5 ml EDTA bloed

Quels résultats pouvez-vous attendre de nous :

1. Confirmation du diagnostic de malaria
2. Identification de l'espèce, parasitémie et stades
3. Test antigène
4. PCR (si exigée pour le diagnostic)

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
 Philippe Gillet
 Annelies Scheirlinck
 Marjan Van Esbroeck
 Lieselotte Cnops
 Peter Konings
 Anne-Mie Feyens
 Agnes D'Hondt
 Pieter Guetens