



WorldMEDSchool
GLOBAL EDUCATION

Malaria Diagnosis

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BILL & MELINDA
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Febrile illness - What do I do?



- ▶ One cannot reliably make a diagnosis of malaria based on clinical criteria (presentation, symptoms, physical exam, or simple lab tests)
- ▶ Malaria, especially *falciparum* malaria, can be rapidly fatal in the non-immune without treatment



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T3: Test, Treat and Track



- ▶ Universal coverage
- ▶ Every suspected malaria case is tested with a quality diagnostic
- ▶ Every confirmed case is treated with a quality assured ACT
- ▶ Every treated case is tracked through timely and accurate surveillance systems
- ▶ Information to guide policy and operational decisions



Test. Treat. Track.

Scaling up diagnostic testing, treatment and surveillance for malaria

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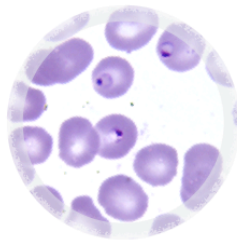
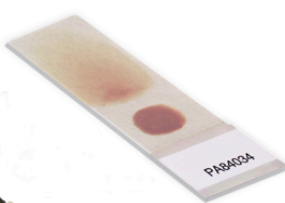
Parasitologic Confirmation is Desirable... when possible and timely



- **Microscopy:**
 - Thick blood films (smears)
 - Thin films (smears)
- **Malaria Rapid Diagnostic Tests (mRDTs):**
 - Antigen capture
- **Molecular (PCR)**
- **Diagnostic services must be:**
 - Accurate
 - Timely
 - Available

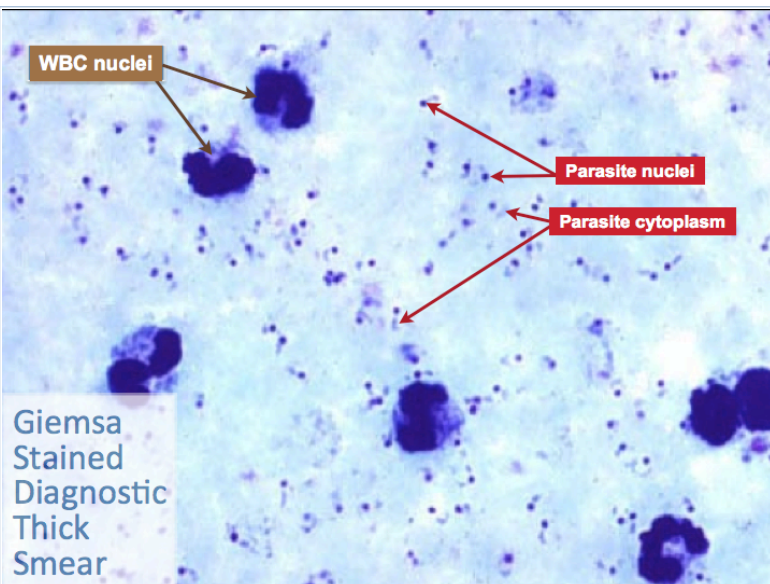
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Microscopy Considered the Reference Standard for the Diagnosis of Malaria

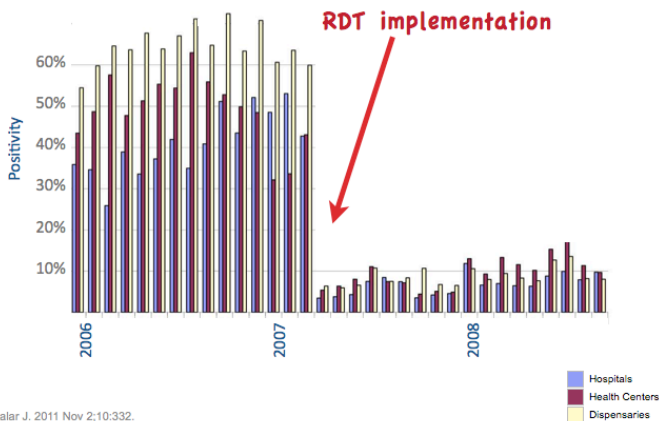


- ➔ Classic diagnostic method in use for > 100 years
- ➔ Yields a wealth of useful diagnostic information

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Positive test results before and after RDT implementation



Consequences of Over Diagnosis of Malaria in SSA



- People get treated for malaria when they don't have it, thus the correct diagnosis is missed or overlooked
- Artemisinin Combination Treatments for malaria are used when not needed
- Confusion

Microscopy for Malaria: The Reality

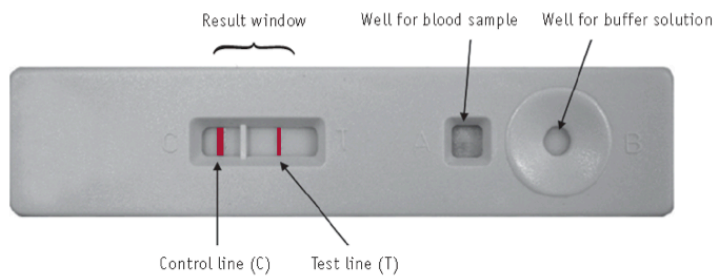


1. Often inaccurate:
 - Inexperienced personnel
 - Poor technique
 - Not familiar with making a thick film
2. Not available
3. Rarely Timely

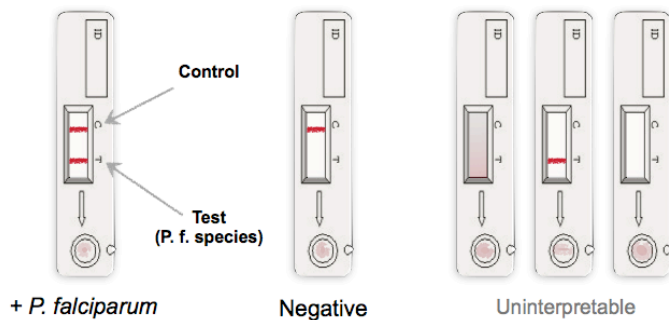


Generic Immunochromatographic Test (ICT) Format

Schematic representation of an RDT cassette



Generic Immunochromatographic Test (ICT) Format



mRDT: Key Points



- RDTs detect antigen (Ag), not parasites
- Assumption that Ag = parasites is not true
- *P. falciparum* peripheral parasitemia does not = parasite burden (sequestration)
- Ag kinetics (clearance, elimination, etc.) may not equal parasite kinetics
- Mixed infections can be confusing



Antigens

- ***P. falciparum* histidine rich protein 2 (HRP2)**
 - Pf asexual and gametocytes
 - Not found in other Plasmodia
- ***P. falciparum* aldolase**
 - Enzyme, most Abs in use are cross reactive to all Plasmodia
- **Species specific parasite lactate dehydrogenase (pLDH)**
 - Pf and Pv

Control line
Plasmodium generic line
P. falciparum



Microscopy for Malaria: The Reality

→ True Positive (TP) =

Smear (+)
mRDT (+)

→ False Positive (FP) =

Smear (-)
mRDT (+)

→ True Negative (TN) =

Smear (-)
mRDT (-)

→ False Negative (FN) =

Smear (+)
mRDT (-)

		Malaria Rapid Diagnostics Test	
		+	-
Microscopy	+	TP	FN
	-	FP	TN

Caveat! Microscopy is usually the comparator method

False
Positive



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False
Negative

Causes of False Negative Test Results



- **Prozone effect (no visible or faint visible lines)**
 - High parasitemias (> 4%)
 - Re-test at 1:10 and 1:100 dilution
- **HRP2 deletions / HRP2 sequence variation**
- **"Faint" lines due to very low antigenemia**

Prozone Effect: False Negative RDT Results

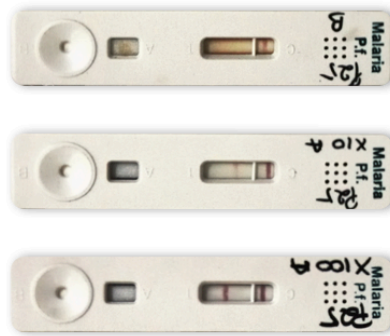


RDT result is negative (-) but patient has malaria:

Fail to treat for malaria when patient has malaria – BAD!

→ **Prozone effect (no visible or faint lines):**

- High parasitemias (> 4%)
- Re-test at 1:10 and 1:100 dilution.



HRP2 Deletions



- **Failure to detect *P. falciparum* HRP2 when infection present**
 - HRP2 gene deletions reported
 - ➔ West African parasites (Mali)
 - ➔ Papua New Guinea
 - ➔ Peru
 - Allelic variation of HRP2 that is not recognized by test capture and detection antibodies



Faint Lines

- **Faint Test lines:**
 - Low peripheral parasitemias = small amount of antigen in the peripheral blood = faint test lines
 - Interpretation difficult (negative vs. positive)
- **Problem exacerbated by:**
 - Poor lighting
 - Poor near vision (presbyopia in age > 40)
 - Impaired vision, inability to focus in the ill patient



Lower Limits of Detection

- **Caveats:**
 - Comparator is peripheral parasitemia as determined by microscopy
 - Appropriate comparator is Ag via quant ELISA
- **Pf HRP2: 10-100 parasites/mcl or 1-10 ng/ml (?)**
- **Best research microscopy @ 5 parasites/mcl**
- **Routine microscopy is 50-100 parasites/mcl**

www.wpro.who.int/sites/rdt

Malaria Rapid Diagnostic Tests

World Health Organization | FIND | TDR | For research on malaria at priority

Home

Making Rapid Diagnosis Work.....

Malaria Rapid Diagnostic Tests (RDTs) assist in the diagnosis of malaria by detecting evidence of malaria parasites in human blood.

This site aims to:

- Provide information and guidance for malaria RDTs to malaria control programmes and health services, organizations and individuals considering the use of malaria RDTs.
- Provide guidance on evaluation of malaria RDTs.
- Provide information to manufacturers and users on WHO-FIND Malaria RDT Evaluation Programme.

Main areas of work:

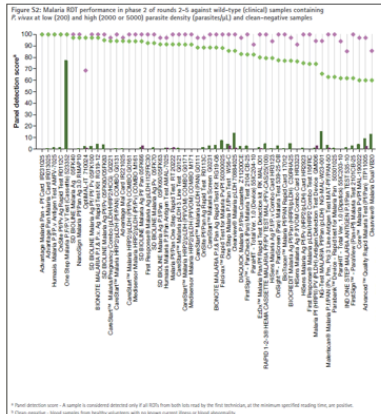
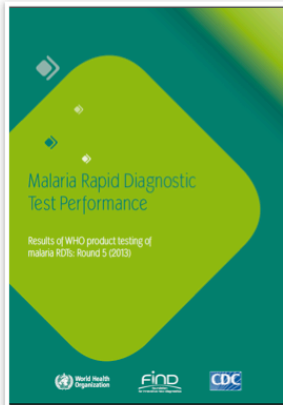
Procurement Implementation Quality Assurance Field Trials

WHAT'S NEW

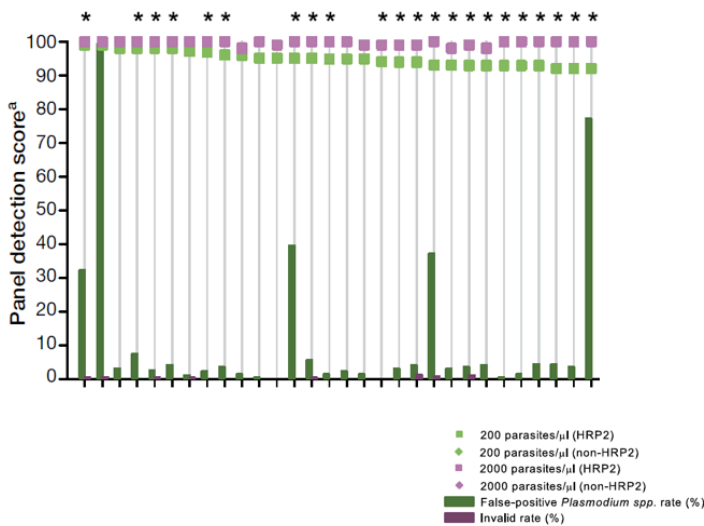
- Expression of interest for Round 4 WHO Malaria RDT Product Testing released
- Interactive RDT Performance Guide to assist in selection of malaria RDTs
- Results of Product Testing Round 2
- Lot Testing
- RDT Training Materials and Job Aids
- Recommendations for Malaria RDTs in Global Fund proposals

Malaria Rapid Diagnostic Test Performance

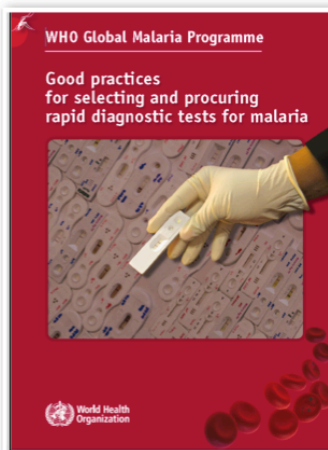
(Round 5, 2013 WHO, FIND, & CDC)



http://apps.who.int/iris/bitstream/10665/128678/1/9789241507554_eng.pdf?ua=1



Malaria Rapid Diagnostic Test Selection and Procurement



[www.finddiagnostics.org/
resource-centre](http://www.finddiagnostics.org/resource-centre)

Limitations of WHO RDT Testing



- Voluntary submission of kits to WHO
- No inspections of the manufacturing facilities
- No ongoing lot submissions
- Testing is analytical
- No clinical trials

WHO Prequalified Malaria Diagnostic Tests



- Two mRDTs are prequalified by WHO - both are Pf HRP2 based tests:
 - SD BIOLINE Malaria Ag P.f. from Standard Diagnostics, Inc. (Korea)
 - Immunoquick Malaria falciparum from Biosynex (France)

- Public Reports available at:

www.who.int/diagnostics_laboratory/evaluations/PQ_list/en



Malaria Diagnosis

