

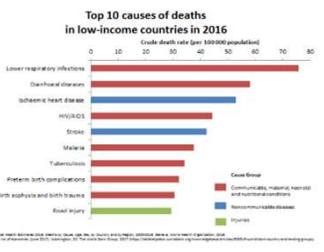


**AAFP GLOBAL HEALTH SUMMIT**  
*Primary Health Care and Family Medicine: Health Equity for All*

**Diagnosing Malaria**  
 Microscopy, RDT's, and the Cost of Misdiagnosis

Andrew Morris, MD

### Why is Malaria diagnosis a big deal?



**Top 10 causes of deaths in low-income countries in 2016**

Cause of Death	Crude death rate (per 100,000 population)
Lower respiratory infections	~75
Diarrhoeal diseases	~65
Ischaemic heart disease	~55
HOUSING	~45
Stroke	~40
Malaria	~35
Tuberculosis	~30
Preterm birth complications	~25
Birth asphyxia and birth trauma	~20
Road injury	~15

- During our session, 50 people will die of malaria.
- 435,000 deaths/year (WHO 2017)
  - 61% under age 5
  - majority (250,000) in Africa

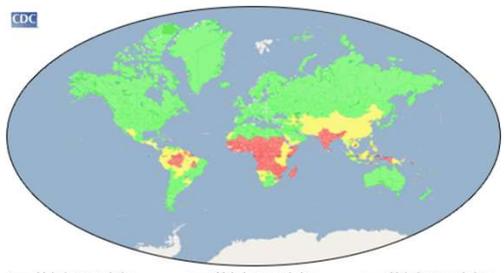
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### No conflicts of interest



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CDC

Legend:  
 ■ Malaria transmission occurs throughout (Red)  
 ■ Malaria transmission occurs in some parts (Yellow)  
 ■ Malaria transmission is not known to occur (Green)

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**Goal:** to accurately rule in or rule out malaria

**Objectives:**

- Know the relative accuracy of available diagnostics for malaria
- Know how to prepare malaria smears for microscopy
- Understand the mechanism and limitations of Rapid Diagnostic Tests
- Understand how to appropriately use RDT's as a diagnostic tool

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### Case – 27 y/o female with fever



27 year old female patient in E. Africa comes to a health post in a rural area located 1 hour by bus to the nearest hospital. She is complaining of fevers, malaise, headache, and nausea after recent spontaneous abortion 2 weeks prior.

T 37.4 C R 32 P 100 BP 102/58 W 53 kg

Exam: Ill and tired appearing, thin habitus, walks on her own power

HEENT: Conjunctival pallor, + icterus, dry mucous membranes

CVS: Mild tachycardia with 2/6 murmur at RUSB

Pulm: No wheezes/rales/rhonchi, + mild tachypnea

Skin: No rashes Abdomen: spleen and liver palpable, smooth borders, mildly-tender

Ext/MSK: No nuchal rigidity, No edema, 2+ pedal pulses

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### The Cost of Overdiagnosis

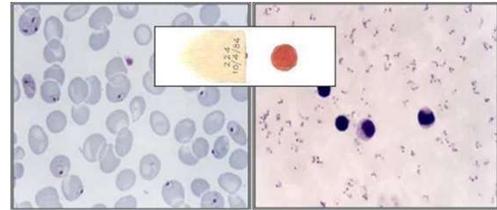
**Table 3** Case fatality by research blood slide result and age among cases with at least one study criterion of severe disease

Age (years)	Total	Slide positive		Slide negative	
		No of cases	No (% of deaths)	No of cases	No (% of deaths)
<2	1855	1016	67 (6.6)	839	82 (9.8)
2-4	996	598	27 (4.5)	398	28 (7.0)
5-14	441	196	21 (10.7)	245	29 (11.8)
≥15	1181	252	27 (10.7)	929	153 (16.5)

Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study

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### Thin Smear      Thick Smear



Parasitic Index:  $\text{Infected RBC's} / \text{total RBC's} \times 100 = \% \text{ Parasitemia}$

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### The Cost of Overdiagnosis

- Overusing costly antimalarial treatments (supply issues)
- Ignoring potentially fatal alternative diagnoses

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### Microscopy - Thin Film, Thick Film

#### Malarial Forms

- Ring form (Early stage)
- Trophozoite stage – (Growth stage) - creates pigment, color varies
- Schizont (Asexual reproduction) (P. vivax, P. malariae)
- Gametocyte (Sexual differentiation) - round or banana shaped

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### Qualities of a Good Test

	PCR	Microscopy	RDT's
Specificity	100%	98%	95%
Sensitivity	100%	89%	>75%
Affordability	\$\$\$	\$	\$
Ease of use	expensive equipment	experienced microscopist	easy
Detection	ssRNA	visual	antigens

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Species	P. Falciparum	P. Vivax	P. Malariae	P. Oval
Ring Stage				
Trophozoite				
Schizont				
Gametocyte				

Zahoor, Jan et al.

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### Rapid Diagnostic Test (RDT)

Malaria antigens present in RBC's  
Add RBC Lysis buffer

Tagged Ab's to the antigen at the indicator line are drawn to the surface if the antigen is present, creating a visible line at the Test Indicator (T).

<https://www.who.int/malaria/areas/diagnosis/rapid-diagnostic-tests/rdt-cassette.jpg>

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### HRP2/3 deleted *P falciparum*

Utilize RDT that looks for HRP2 and pLDH

1. pfHRP2 + pfLDH (pf+pf)
2. panLDH - will not identify species
3. pfHRP2 + panLDH
4. pfHRP2/pfLDH + panLDH - not yet available

Rahmeti Balaghalah M, Zarean M, Atzal Aghaee M, Shamsian SA, Mirzadadi H, et al. Comparison of pHRP2/pfLDH RDTs with Light Microscopy in a Low Prevalence Setting in Southwestern Iran, Sistan and Baluchistan: Due to Implementation of Malaria Elimination Program. Int J Infect. 2018 ; 5(1):e12286. doi: 10.5812/ij.12286.

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### Malaria antigens

Plasmodium Antigen	Species Detection	Limitations
HRP2/3	<i>P falciparum</i>	HRP2/3 deletions, Persistent antigenemia
Pf-pLDH	<i>P falciparum</i> , including HRP2/3 deletions	Lower sensitivity at lower parasite density
pLDH (pan)	All species or species specific	Lower sensitivity
pAldolase (pan)	All species	Lower sensitivity

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### RDT False Negatives (reduced sensitivity)

1. Parasite burden insufficient to produce + result.
2. Damaged RDT - packaging, heat exposure, moisture, expiration
3. RDT not designed to detect plasmodia species causing the illness

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### Case – 27 y/o female with fever

Which test do you want to use?

1. Look up epidemiology of malaria in the region: 99.7% falciparum in sub-saharan Africa. Report of HRP2 deletions in the region.
2. Utilize the FIND Interactive Guide to determine the best RDT for your region. <http://www.rdt-interactive-guide.org/>

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### RDT False Positives (Reduced Specificity)

1. Antigen persistence (pLDH less persistent than HRP2)
2. Presence of gametocytes not causing illness
3. Presence of parasitemia not causing illness in patient with high immunogenicity
4. Other substances in blood causing false +

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**Case – 27 y/o female with fever**



RDT - pfHRP2 + pLDH (pf+pan)

Test is negative

What do you recommend next?

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**The Use of Malaria Rapid Diagnostic Tests**



*Misdiagnosis of malaria results in significant morbidity and mortality. Rapid, accurate and accessible detection of malaria parasites has an important role in addressing this, and in promoting more rational use of increasingly costly drugs, in many endemic areas. Rapid diagnostic tests (RDTs) offer the potential to provide accurate diagnosis to all at-risk populations for the first time, reaching those unable to access good quality microscopy services.*

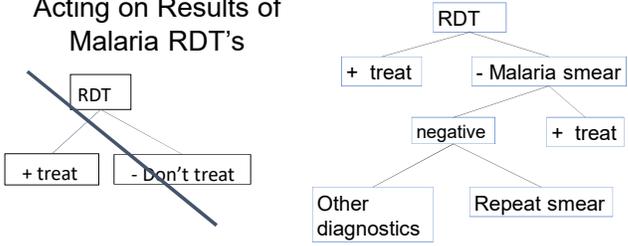
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**The impact of providing rapid diagnostic malaria tests on fever management in the private retail sector in Ghana: a cluster randomized trial.**

- > Of those presenting to a chemical shop at the community level with fever and symptoms of non-severe malaria, 70-80% had negative slides.
- > 32% intervention (RDT) group with negative results received malaria tx
- > 88% control group (clinical dx) with negative results received malaria tx
- > 88-89% of pts with positive results received malaria tx in both groups

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**Acting on Results of Malaria RDT's**



```

    graph TD
      RDT --> RDT_Pos[+]
      RDT --> RDT_Neg[-]
      RDT_Pos --> Treat_Pos[+ treat]
      RDT_Neg --> Malaria_Smear[- Malaria smear]
      Malaria_Smear --> Smear_Pos[+]
      Malaria_Smear --> Smear_Neg[-]
      Smear_Pos --> Treat_Smear[+ treat]
      Smear_Neg --> Other_Diag[Other diagnostics]
      Smear_Neg --> Repeat_Smear[Repeat smear]
  
```

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**Case – 27 y/o female with fever**



Negative RDT

Fever

Recent SAB

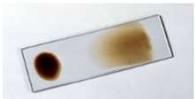
Pt unlikely to go to hospital immediately

Perform blood smear

Smear is + for falciparum malaria. She is treated with ACT and recommended to go to the hospital if she does not improve.

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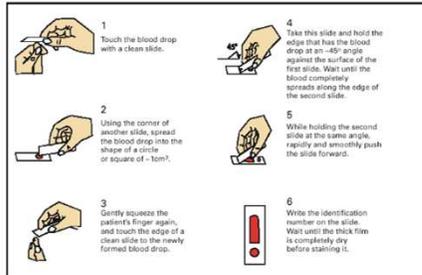
**Procedure for Blood Films**



1. Collect blood for Thick and Thin Smears x 2
2. Label Slides
3. Air Dry - for rapid microscopy, air dry thin film on separate slide
4. Fix Thin Smear with methanol for 15-30 seconds
5. If not staining slides immediately, rinse thick smear with H2O to hemolyze rbc's
6. Stain
  - a. Giemsa - soak in 2.5% Giemsa for 45-60 minutes, then rinse in buffer (thin-5 dips, thick -5 min)
  - b. Wright-Giemsa
  - c. Leishman
  - d. Fields
7. Wash with deionized water
8. Look for blue cytoplasm and red chromatin dot intracellularly

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Figure A-2. Preparation of a thin and a thick blood film on the same slide.



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## Summary

- A clinical diagnosis of malaria is wrong much of the time even in endemic areas.
- The cost of being wrong is high.
- RDT's are useful technology that increase the specificity of diagnosis.
- RDT's are species/region specific and must be quality controlled.
- Use RDT's and be prepared for next steps when the RDT is negative.

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