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.

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

Scaling up diagnostic testing, treatment and surveillance for malaria
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

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

Giemsa Stained Diagnostic Thick Smear

- WBC nuclei
- Parasite nuclei
- Parasite cytoplasm
**Problems with Microscopy as the Diagnostic Reference Standard**

- **Complex Procedure:**
  - Requires acquired technical skills, quality equipment and reagents, and continuing education

- **Malaria endemic areas:**
  - Microscopy often not available
  - Considerable variability in the quality of smears and interpretations
  - Training and microscope maintenance

- **Malaria non-endemic areas:**
  - Difficult to maintain proficiency
  - Not available where needed most = point of care = EMD

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**Microscopic Diagnosis of Malaria**

**False Positive**
- Inexperienced microscopist
- Not familiar with thick smear technique
- Artifacts on slide
- Poor quality smears

**False Negative**
- Low parasitemias
- Misdiagnosis
- Pf (+) but called Pv
- Mixed Pf / Pv

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**Over Diagnosis of Malaria in Tanzania**

- Of 53% of blood slides positive in routine laboratories, only 2% were positive by expert microscopy
- Sensitivity of routine microscopy was 71.4% and specificity was 47.3%
- Positive and negative predictive values were 2.8% and 98.7%, respectively
- Median parasitemia was only three parasites per 200 white blood cells (WBC) by routine microscopy compared to 1226 parasites per 200 WBC by expert microscopy
- The sensitivity and specificity of RDTs using expert microscopy as reference were 97.0% and 96.8%
Positive test results before and after RDT implementation

![Graph showing 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

- Result window
- Well for blood sample
- Well for buffer solution
- Control line (C)
- Test line (T)

**Generic Immunochromatographic Test (ICT) Format**

- Control
- Test (P. f. species)
- + P. falciparum
- Negative
- Uninterpretable

**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

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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
**RDT False Positive Result**

- Inappropriate anti-malarial treatment
- Delayed diagnosis and appropriate treatment or management of other important causes of undifferentiated fever
  - Typhoid fever
  - Dengue
  - Shigellosis
  - Leptospirosis
  - Rickettsia

**Causes of False Positive Test Results**

- IgM antibodies:
  - Rheumatoid Factor
  - Human African Trypanosomiasis
- Time at which test lines interpreted

**Why False Negative Test Results are Important**

Failure to identify a potentially life threatening and easily treatable infection
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**

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Prozone Effect: False Negative RDT Results

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

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

http://apps.who.int/iris/bitstream/10665/128678/1/9789241507354_eng.pdf?ua=1

![Graph showing panel detection score results.](image)

Malaria Rapid Diagnostic Test Selection and Procurement

Good practices for selecting and procuring rapid diagnostic tests for malaria

[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)
  - Immunochip Malaria falciparum from Biosynex (France)

- Public Reports available at:
  www.who.int/diagnostics_laboratory/evaluations/PQ_list/en