Rapid Diagnostic Tests in Microbiology
(PAs & PHs)

I. Afeke
UHAS
Objective: Upon completion of this topic, the student should be able to:

- What a rapid diagnostic test (RDT) is
- When a RDT should be used
- How a RDT should be used
- Who should use a RDT
- Advantages and disadvantages of RDTs
What are Rapid Diagnostic Tests?

• RDT or also known as Rapid Point-of-Care Test

• Diagnostic tests that we obtain **Results** in **minutes** or at most 1-2 hours

• Include “point of care” (for doctor, PAs and nurses) and “walk away” tests (home tests)

• Can also be used in microbiology labs by Lab. Technologists
Other Laboratory based Diagnostic Tests

➢ Requires time:

• Bacterial culture: 24-72 hours

• Identification: 4-24 hours

• Antibiotic susceptibility testing: 24 hours...

• ELISA: 2-4 hours, requires several samples to decrease the cost

➢ Requires trained people
Overall Comparison

• Other Diagnostic Tests

• RDTs

Can you imagine the challenges of shrinking a huge laboratory filled with people and equipment onto a single chip the size of a matchbox?
The Need for RDTs

• The demand for fast, easy-to-use and sensitive diagnostic tests for microbial infections is on the rise (great opportunities for researchers)

• Disease epidemic requirements for biological confirmation at peripheral level (is not possible or feasible without RDTs)

• Rapid diagnostics for improving the quality of care for patients with suspected infections:
  - Diagnostics influence 60-70% of health care decision making but account for less than 5% of hospital costs (Lewin report 2006)

• Improve antibiotic targeting to only those who will benefit, thus reducing overuse:
  - The commonest reason for prescribing antibiotics in the community is acute cough, and these prescriptions virtually never benefit patients (Butler et al, BMJ 2009)
• Enhance surveillance of pathogens and infectious diseases:
  - e.g. H1N1 flu pandemic
    Community-Acquired Pneumonia (CAP)

• Support rapid initiation and cessation of treatment:
  - Sepsis is associated with 7% increased mortality for every hour delay in the administration of appropriate antibiotics (Kumar et al, CCM 2006)
  - Ventilator-Associated Pneumonia (VAP)

• Decrease the size and cost of antibacterial clinical trials:
  - We URGENTLY need new antibiotics (ECDC/EMA report 2009)
Principles & how to perform RDTs

Methods:

❖ Antigen detection
  - from the microbes (on it or in it)

❖ Antibody detection
  - produce by host (humans) who has been infected by microbe
  - seroconversion (negative to positive)

❖ Molecular detection
  - lysis of the microbe to release its DNA
(1) Antigen Detection

Detects bacterial, viral or parasite antigen (surface antigen, soluble antigen) or toxin in biological fluids (CSF, blood, urine)

Primary techniques:

- Direct agglutination: slides, cards
- Latex agglutination: slides, cards
- Immunochromatography: dipsticks
Latex agglutination test: Principle

Latex beads
(= polystyrene particles)

bacterial Ag

Antibodies specific to
Bacterial polysaccharide Ag

Source: WHO meningitis workshop Ouagadougou Sept 2004
How to perform the test

1. Take patient’s sample: Whole Blood, Serum, plasma, Urine etc.
2. Put a drop on the card
3. Add a drop of antiserum to it and mix
4. On the same card (different side) add a drop of antiserum and then a drop of sterile saline (serves negative control)
5. Swirl for about 2 minutes
6. Look for agglutination/precipitation
Dye-labelled antibody, specific for target antigen, is present on the lower end of nitrocellulose strip or in a plastic well provided with the strip.

Antibody, also specific for the target antigen, is bound to the strip in a thin (test) line, and either antibody specific for the labelled antibody, or antigen, is bound at the control line.
Blood and buffer, which have been placed on strip or in the well, are mixed with labelled antibody and are drawn up strip across the lines of bound antibody.

**Parasite antigen (AG.)**
Captured by labeled AB.

Source: [http://www.wpro.who.int/rdt](http://www.wpro.who.int/rdt)
Malaria P.f. RDT Results

NEGATIVE RESULTS

Wait 15 minutes before reading results.

POSITIVE RESULTS

INVALID RESULTS *

* No Control Lines (repeat tests)

Source: http://www.wpro.who.int/rdt
(2) Antibody (Ab) Detection

Requires seroconversion detection:

- IgG titer elevation not possible with RDT (= qualitative)
- IgM detection (after IgG elimination or IgM capture)

Main techniques:

- Direct agglutination (red cells + antigen, latex + antigen)
- Agglutination inhibition
- Immunodot
- Immunochromatography
(3) Molecular detection

Real-time PCR?

- DNA extraction < 1 h
- Simultaneous amplification and detection < 2 h
- Cost +++
Advantages of RDTs

✓ Easy to use, minimal training
✓ Rapid – same day results possible
✓ Shelf life up to 1-2 years without refrigeration
✓ Limited/no instrumentation; can be performed at the periphery of health systems without laboratory or electricity
✓ Some tests as accurate as reference-level laboratory tests
Disadvantages

• Cost per test more than traditional tests

• Some have limited shelf lives therefore increased demands on procurement and distribution

• Mainly produce only "yes/no" answers

• Could require subjective interpretation (reader variation)

• Rapid tests can be less sensitive or less accurate compared to existing tests