Rapid Tests for Common Infections

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Overview

- What are rapid tests and why are they needed?
- How do these tests work?

Point of Care Tests

- Medical diagnostic testing performed outside the clinical laboratory, in close proximity to where the patient is receiving care
  - Typically performed by non-laboratory personnel, with results used for clinical decision making
- More commonly referred to as Rapid Diagnostic Tests in the infectious disease literature
- Most critical elements of POCT are:

  (1) rapid turn-around and communication of results to guide clinical decisions

  (2) completion of testing and follow-up action in the same clinicaencounter

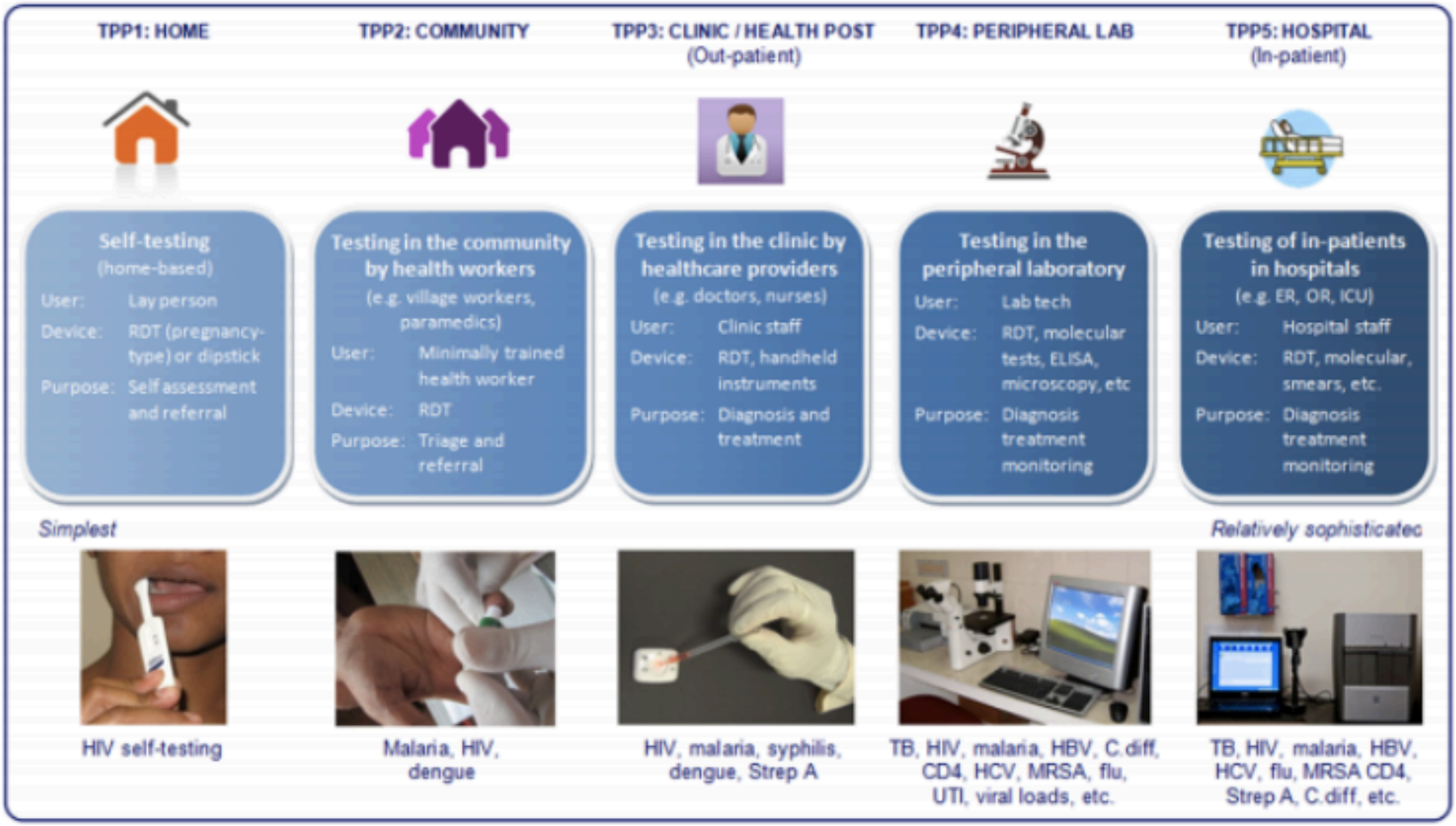


Figure 1. Diversity of target product profiles, users, and settings within the spectrum of POC testing. HBV, hepatitis B virus; HCV, hepatitis C virus; UTI, urinary tract infection; MRSA, methicillin-resistant staphylococcus aureus; C. diff, clostridium difficile; RDT, rapid diagnostic test; Strep A, group A streptococcus.
doi:10.1371/journal.pmed.1001306.g001

Pai NP, Vadnais C, Denkinger C, Engel N, Pai M (2012) Point-of-Care Testing for Infectious Diseases:Diversity, Complexity, and Barriers in Low- And Middle-Income Countries. PLoS Med 9(9): e1001306. doi:10.1371/journal.pmed.1001306

Why are POCTs needed?

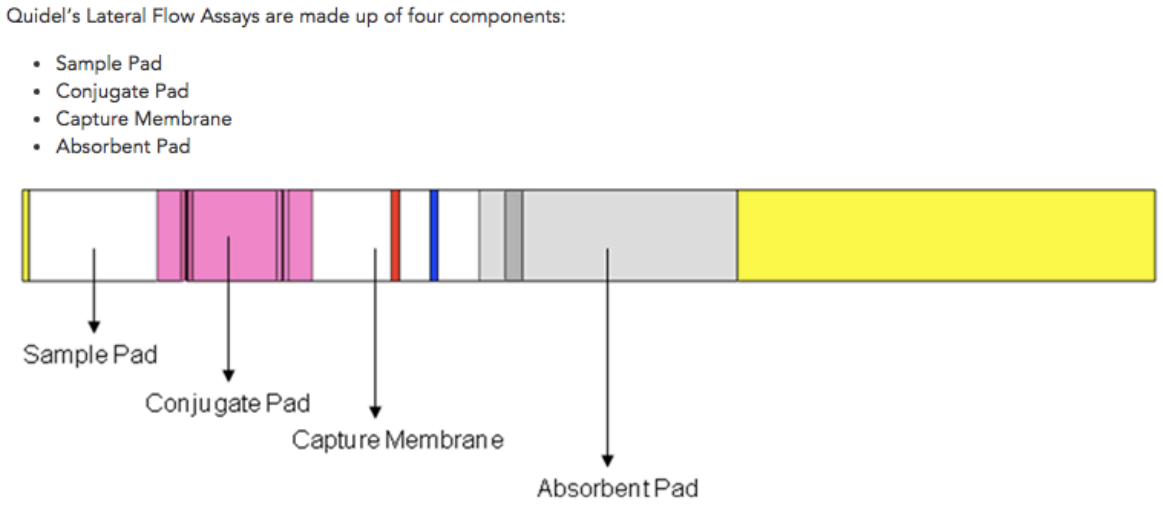
- Specially useful in resource-limited countries
  - Quick and easy to perform (10 mins to 2 hrs), require little or no additional equipment
  - Performed by trained healthcare professionals rather than pathology staff
  - Designed for use with individual or a limited number of samples, which make them more economical then ELISAs
  - Possibility to store at room temperature for extended period of time
- Useful in special situations
  - Outbreaks where a rapid result is required for patient management and infection control
  - Outpatient settings, where patient might not wait or return for result if it takes a few hours to be processed

POCT: Test Categories

1. Antigen detection (enzyme immunoassay)
2. Molecular etection (NA probes and nucleic acid amplification)
3. Rapid biochemical tests (nitrite or leukocyte esterase tests on urine dipsticks)
4. Direct microscopy of specimens using microbiologic stains, including Gram stain
5. Serologic testing

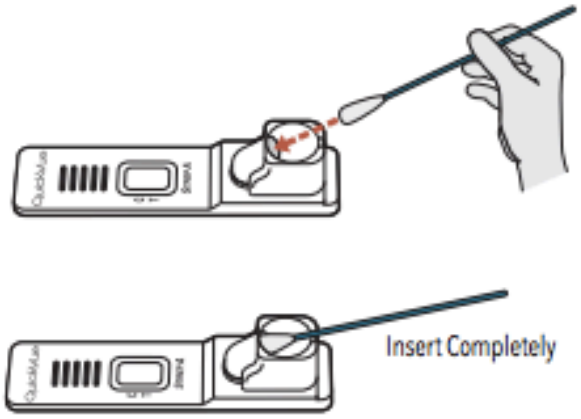
Test Formats (1)

- Most common format of a POCT kit in microbiology i→ antigen (or antibody) capture method using a lateral-flow ICT system
  - Enzyme immunoassay: nitrocellulose strip embedded with complementary antibody(or antigen) to the protein of interest, conjugated to a colloidal metal or colored dye

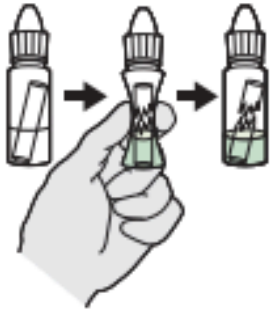


Lateral Flow Immunoassay for GAS

1 **MUST** use the swabs provided in the kit

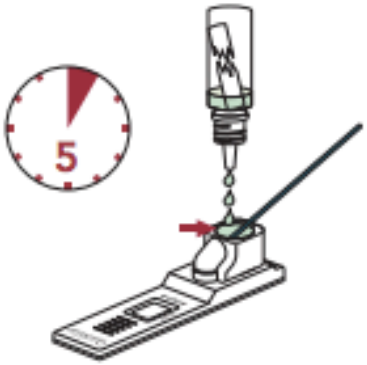


2 Squeeze **ONCE** to break.



3

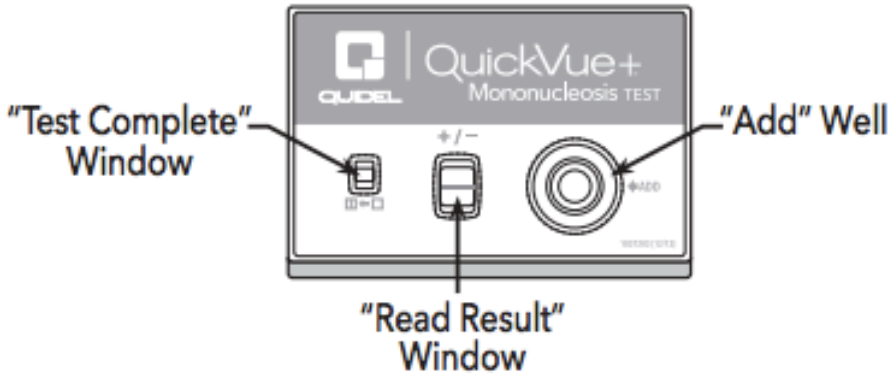
4 Holding bottle vertically, quickly fill the chamber to the rim (approximately 8 drops)



* *Look closely! This is a positive result. Even if you see a very faint, pink Test Line and a blue Control Line, you must report the results as POSITIVE. The positive test line is usually very prominent, but test line intensity can vary.*

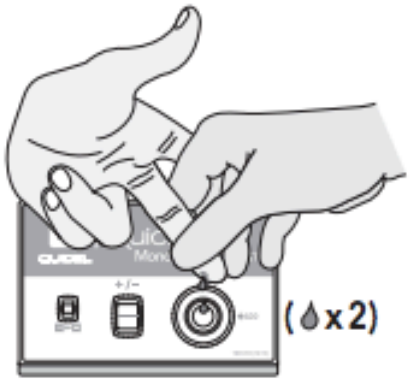
If liquid has not moved across the Result Window in 1 minute, completely remove the swab and re-insert.

Color Immunochromatographic Assay for Infectious Mononucleosis



Hanging Drop Procedure

Add 2 hanging drops of fingertip blood directly to the center of the "Add" Well.



Dipstick Test for RSV

Nasopharyngeal Aspirate or Nasal/Nasopharyngeal Wash Test Procedure

1. Just before testing, add Extraction Reagent to the test tube up to the **fill line** (250 µL).

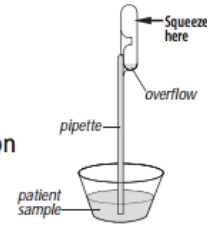
Note: Too little or too much of the Extraction Reagent may cause erroneous results

2. To fill the pipette with the sample*:

a) FIRMLY squeeze the top bulb.

b) Still squeezing, place the pipette tip into the liquid sample.

c) With the pipette tip still in the liquid sample, release pressure on bulb to fill the pipette (extra liquid in the overflow bulb is OK).



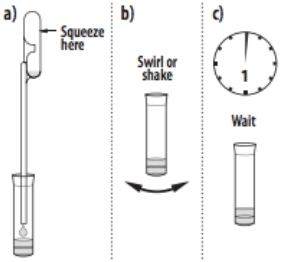
***NOTE:** The pipette is designed to collect and dispense the correct amount of liquid sample.

3. To add the sample to the test tube:

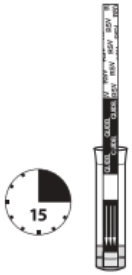
a) Firmly squeeze the top bulb to add the sample in the pipette to the test tube with the reagent. The correct amount will be added, even though the overflow bulb will not empty. Discard the pipette.

b) Swirl or shake the tube to mix.

c) Wait one (1) to two (2) minutes to allow the mixture to react.



4. Place the Test Strip into the tube with the arrows pointing down. Do not handle or remove the Test Strip for fifteen (15) minutes.



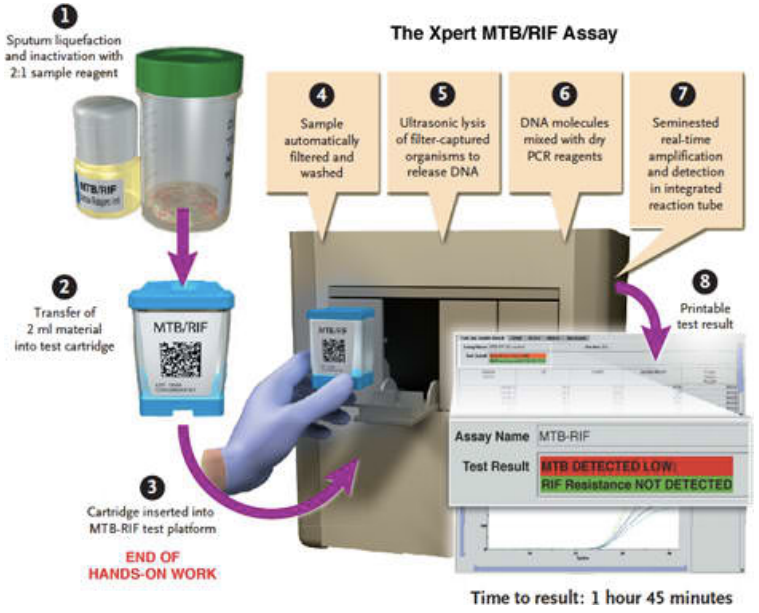
5. Remove the Test Strip, and read the result according to the Interpretation of Results section. Some positive results may appear sooner than 15 minutes.

Dipstick Test for Malaria

FIG. 1. Three examples of a malaria rapid immunochromatographic test, in which results are weakly positive (left), clearly positive (middle), and negative (right). C, positive control band; P, *Plasmodium* species band; Pf, specific *P. falciparum* band.

Test Formats (2)

- Point-of-care test based on the detection of microbial nucleic acids
  - Molecular POCT methods have the potential to provide **greater sensitivity and specificity** than immunological assays



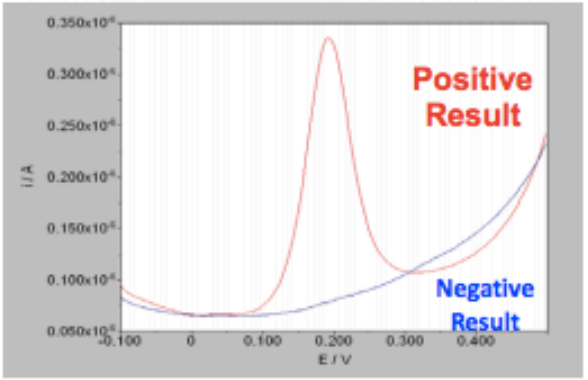
Test Formats (2)

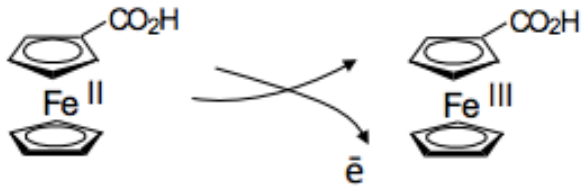
- Challenge of point-of-care testing: creation of smaller, easily portable devices

- GeneXpert Omni
- Size: 23 cm height, weight 1kg
- PCR-based cartridge test
- Battery-operated, wireless and web-enabled

Atlas Velox POC Test for Chlamydia

- **User simply adds sample to card**
- **All other functions performed by system (on card)**
  - **DNA extraction**
  - **PCR amplification**
  - **Detection of target**
- **Perform test & treat in single clinic appointment**
  - **Rapid results in 20 minutes**
- **Principle of Detection**
  Electrochemical label released from probe hybridised to target by nuclease enzyme
  - **Nuclease double strand specific, so no label release in absence of target**
  - **Voltage applied to carbon electrode**
  - **At a known potential the electrochemical label oxidises generating measurable current**

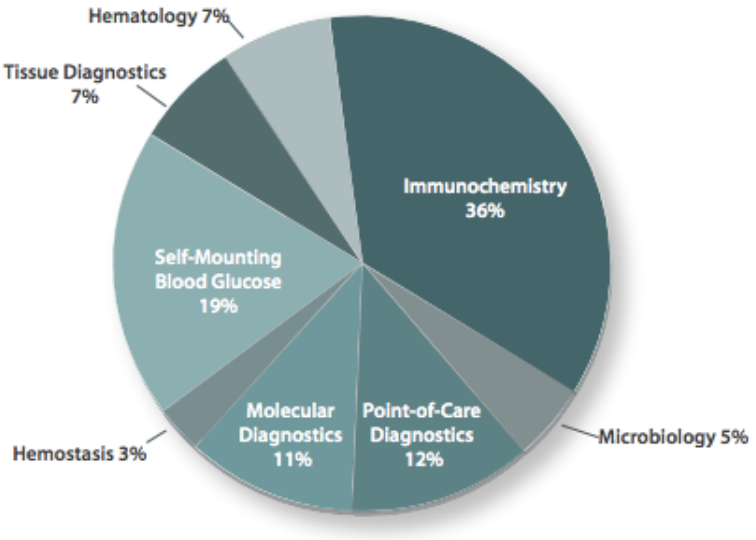




What is in the market?

- Currently, the number of infectious disease POC tests that are approved for use in the United States is limited and focuses on a small set of common clinical conditions.
- Tests cleared by the Food and Drug Administration exist only for HIV, HCV, influenza, RSV, EBV, Group A Streptococcus, adenovirus, Helicobacter pylori, trichomoniasis, bacterial vaginosis, and Borrelia burgdorferi

Figure 1. Global In Vitro Diagnostics Market 2012



CLIA –Waived POCTs, US

CLIA-Waived FDA-Approved POC Tests for Infectious Diseases

Group A Streptococcus: This test, used to detect the presence of Group A Streptococcus in cases of pharyngitis, relies on detection of the bacterium's antigen on a throat swab. It is manufactured by several companies. A positive result on this test would prompt antibacterial therapy for treatment of an acute episode and prevention of rheumatic fever, a serious sequel of untreated infection. Because of its suboptimal sensitivity, culture confirmation is recommended for negative tests in children and adolescents who are at higher risk for both Group A Streptococcus pharyngeal infection and rheumatic fever.

Influenza (A and B subtypes): Rapid influenza antigen detection tests are available from several manufacturers and are performed on nasal or nasopharyngeal swabs. A positive result on this test would prompt initiation of antiviral treatment to ameliorate the symptoms of influenza, possibly prevent serious complications, and potentially reduce contagiousness. Additionally, a positive result for a hospitalized patient will trigger infection control procedures to prevent nosocomial spread of the virus. A secondary benefit of this test is that a positive result, in the setting of an upper respiratory tract infection, can obviate the desire to dispense an antibacterial for a viral illness. Because of the poor sensitivity of these tests, a negative result cannot be relied on.

HIV: HIV POC testing relies on the detection of antibodies against HIV in either saliva or blood. A positive result on this test, while still requiring confirmation with a second type of test, will result in several actions that include: changes in treatment algorithms, changes in risk-taking behavior, initiation of antiviral therapy, and reporting to government health authorities. A limitation of this test is that a short window exists during which anti-HIV antibodies are not detectable with current CLIA-waved POC technology, and other non-POC (PCR or antigen detection) tests would be necessary. A home saliva HIV testing kit also is available.

Hepatitis C virus: POC testing for hepatitis C relies on the detection of antibodies against the virus in blood samples. A positive result on this test would result in linkage to care, counseling on risk-reduction activities, and reporting to government health authorities.

Bacterial Vaginosis: There are 2 types of CLIA-waived tests that are used to diagnose this condition. One test relies on the detection of alterations in the vaginal chemical milieu (pH and amines) induced by the culprit pathogens. Another relies on the detection of enzymatic activity by the culprit pathogens. Both tests are performed on vaginal secretions. A positive result prompts antibacterial therapy.

Respiratory Syncytial Virus (RSV): RSV testing relies on the detection of RSV antigens in nasopharyngeal samples. A positive result on this test does not usually result in the administration of antiviral therapy but does prompt infection control measures in hospitalized patients and diminishes the tendency to prescribe antibacterial therapy for a viral upper respiratory infection. A negative result on the test, because of suboptimal sensitivity, cannot be relied on.

Epstein Barr Virus (EBV): The use of EBV testing is primarily conducted to identify EBV-caused infectious mononucleosis. This test relies on the detection of antibodies induced by the virus in blood. A positive result on this test would prompt counseling regarding risk-reduction activities and prevent prescription of antimicrobial therapy for the viral condition. However, the sensitivity of the test allows for false negative results to occur.

Trichomoniasis: Testing for trichomoniasis relies on an antigen detection test employed on vaginal secretions. A positive result on the test would prompt antimicrobial therapy coupled with risk-reduction counseling activities.

Adenovirus: An antigen detection method to detect the presence of viral antigen in tears is used in this test. A positive result would diminish the likelihood that antibacterial therapy would be employed in this viral illness.

TABLE I. Indication and performances of commonly used point-of-care (POC) tests

Test/pathogen	Type of test	Sample	Indication	Performances	Comment	Ref.
Group A streptococcal rapid test	EIA	Pharyngeal swab	Sore throat	Sensitivity: 53–99% Specificity: 62–100%	Confirmation of negative swabs with culture may be unnecessary for adults	[19]
Pneumococcal antigen	ICT	Urine (pleural fluid, CSF)	Severe pneumonia (empyema, meningitis)	Sensitivity: 66–70% Specificity: 90–100%	Better sensitivity for severe and bacteraemic pneumonias	[25–27]
Legionella antigen	ICT	Urine	Severe pneumonia/risk factors for legionellosis	Sensitivity: 76% Specificity: 99%	Only serotype 1 reliably detected	[34]
Group B streptococci	POC test–PCR	Vaginal swab	Peripartum detection of colonization	✓ Sensitivity: 94–97% Specificity: 96–100%	Performs better than late antenatal screening	[41,42]
MRSA carriage detection	POC test–PCR	Nasal swab	Risk factors, screening	✓ Sensitivity: 86–94% Specificity: 93–95%	PCR-positive and culture-negative samples consistent with many circumstances	[57]
Clostridium difficile toxin detection	ICT	Stool	Antibiotic-associated diarrhoea	Sensitivity: 49–80% Specificity: 95–96%	Notably less sensitive than cultures	[58,59]
Chlamydia antigen	ICT	Vaginal swab, urine	Screening, suspicion of PID	Sensitivity: 83% Specificity: 99%	May lead to treat more infected patients	[48]
Rapid malaria test	ICT	Blood	Fever in returning traveller	Sensitivity: 87–100% Specificity: 52–100%	Sensitivity better for *Plasmodium falciparum* (panmalarial tests)	[54,55]
Giardia lamblia rapid diagnosis	EIA	Stool	Diarrhoea, especially for returning travellers	Sensitivity: 58–98% Specificity: 97–98%	May perform comparably to microscopic examination of stools	[60,61]
RSV antigen	ICT	Nasopharyngeal swab	Viral symptoms, especially during the winter season	Sensitivity: 59–97% Specificity: 75–100%	Lower viral load explains poorer performance in adults	[62]
Influenza rapid test	ICT	Nasopharyngeal swab	Flu-like symptoms	Sensitivity: 20–55% Specificity: 99%	Low sensitivity; probably not helpful during outbreaks; lower in adults	[12,63]
Rotavirus antigen	ICT	Stool	Diarrhoea (children)	Sensitivity: 75–99% Specificity: 95%	May be coupled with adenovirus detection	[64,65]
Adenovirus antigen	ICT	Stool	Diarrhoea	Sensitivity: 22% Specificity: 84%	Poor performance	[65]
HIV rapid test	ICT	Blood (oral fluid)	Screening, prevention of vertical transmission	✓ Sensitivity: 99–100% Specificity: 99–100%	Performance comparable to standard tests	[66]
Enterovirus	POC test–PCR	CSF	Meningitis	✓ Sensitivity: 97% Specificity: 100%	Allows rapid discharge of positive patients	[9,67]

CSF, cerebrospinal fluid; EIA, enzyme immunoassay; HIV, human immunodeficiency virus; ICT, immunochromatographic test; MRSA, methicillin-resistant *Staphylococcus aureus*; PID, pelvic inflammatory disease; RSV, respiratory syncytial virus.

Clerc O et al. Routine use of point-of-care tests: usefulness and application in clinical microbiology
Clin Microbiol Infect 2010; 16: 1054–1061

TABLE 1

Unmet needs for point-of-care (POC) tests in the developing world

	Current diagnosis	Unmet need for POC test
Syndromes/conditions		
Acute lower respiratory infections	Syndromic management using Integrated Management of Childhood Illness algorithms	Test/biomarker to distinguish between bacterial and viral pneumonia
Febrile illness in children	Presumptively treat for malaria in areas of high endemicity	Multiplex POC test for common causes of fever
Sexually transmitted infections, including HIV	Syndromic management for patients presenting with symptoms; POC tests to screen for HIV and syphilis	POC test for genital chlamydial and gonococcal infections; POC test for paediatric diagnosis of HIV; POC test for CD4 and viral load
Antenatal care	POC test for HIV; haemoglobin POC test for anaemia	Multiplex POC test for screening HIV, malaria, syphilis, and anaemia
Diseases		
Malaria	Rapid antigen detection tests	
Tuberculosis	None	Test for active tuberculosis and antimicrobial susceptibility
Human African trypanosomiasis	None that works well	POC test for staging disease; POC test of cure
Visceral leishmaniasis	POC serological test works well in India but not in Africa	POC test of cure

HIV, human immunodeficiency virus.

Peeling R et al. Point-of-care tests for diagnosing infections in the developing world. Clin Microbiol Infect 2010; 16: 1062–1069

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POCT to guide antibiotic prescription for ARI

- One strategy for reduction of antibiotic use in primary care is by use of a point-of-care biomarker, which can be used as a surrogate marker of infection
- Six studies (n=3284) through Jan 2014, patients with ARI from primary care settings. C-reactive protein used as biomarker
- Results:
  - The only point-of-care biomarker currently available to primary care identified in the review was CRP
  - A reduction in antibiotic use is likely to be achieved → due to differences in study designs, not possible to obtain a precise effect estimate of reduction
- Results (continued)
  - No evidence for
    - Longer time to recovery
    - Longer duration of ARI
    - Lower level of patient satisfaction
    - Increased number of re-consultations
- More precise effect estimate is needed to assess the costs of the intervention and compare the use of a point-of-care biomarker to other antibiotic-saving strategies

Aabenhus R, Jensen JUS, Jørgensen KJ, Hróbjartsson A, Bjerrum L. Biomarkers as point-of-care tests to guide prescription of antibiotics in patients with acute respiratory infections in primary care. Cochrane Database of Systematic Reviews 2014, Issue 11. Art.No.: CD010130. DOI: 10.1002/14651858.CD010130.pub2.

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Rapid viral diagnostics for febrile ARI in children at the ED

- To determine if the use of a rapid viral detection test for children with ARI in EDs changes patient management and resource use. Rapid viral testing coud reduce antibiotic use, rate of ancillary testing and length of ED visits
- Studies retrievable as of July 2014, including 759 participants
- Results:
  - Rapid viral testing resulted in a trend toward decreased antibiotic use in the ED → not statistically significant
  - Lower rates of chest radiography
  - No effect on length of ED visits, blood or urine testing
- There is insufficient evidence to support routine rapid viral testing to reduce antibiotic use in pediatric EDs

Doan Q, Enarson P, Kissoon N, Klassen TP, Johnson DW. Rapid viral diagnosis for acute febrile respiratory illness in children in the Emergency Department. Cochrane Database of Systematic Reviews 2014, Issue 9. Art. No.: CD006452. DOI: 10.1002/14651858.CD006452.pub4.

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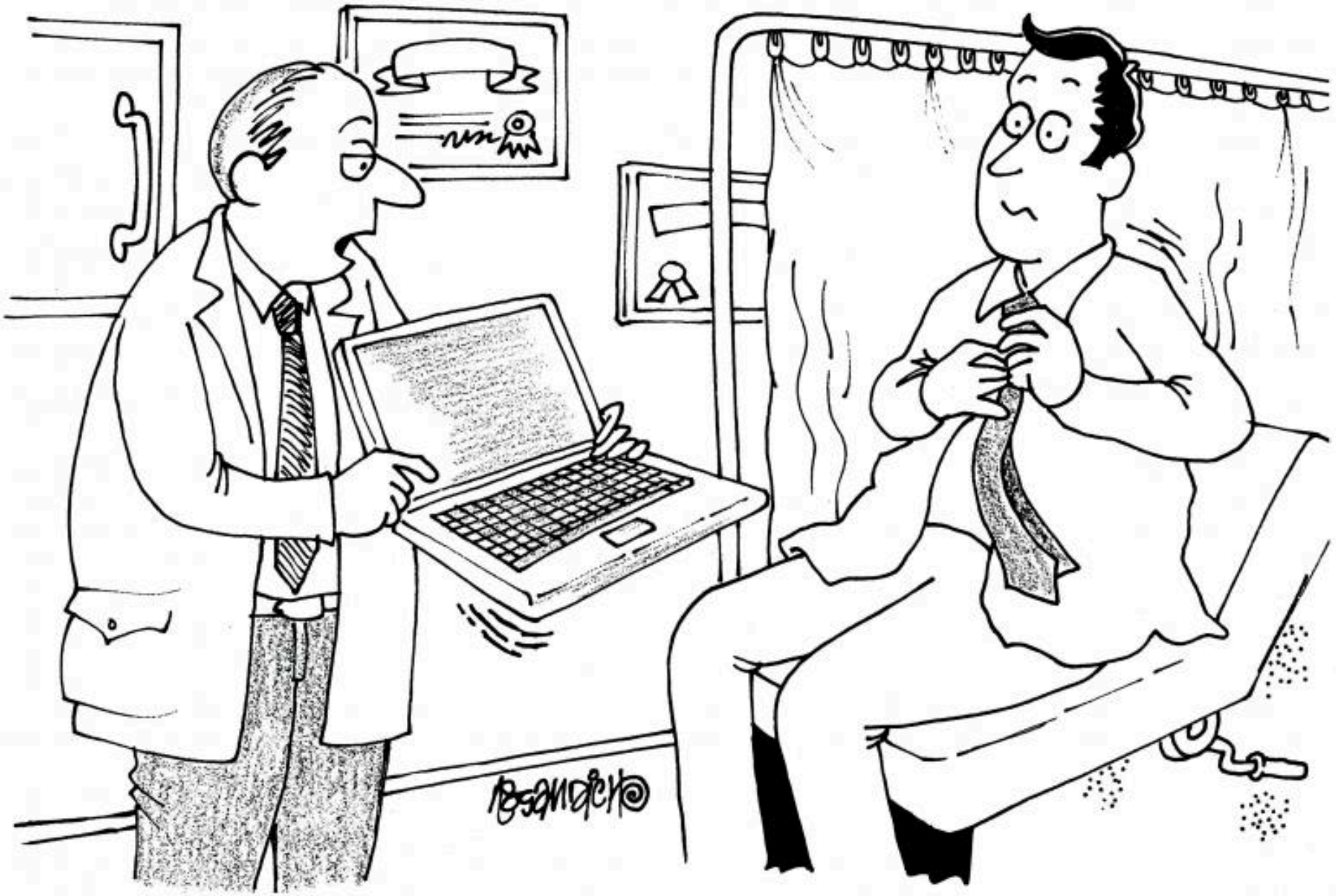
Rapid tests in malaria

- To evaluate whether introducing RDTs into algorithms for diagnosing and treating people with fever improves health outcomes, reduces antimalarial prescribing, and is safe, compared to algorithms using clinical diagnosis.
- Seven trials (N=17,505) with fever or reported history of fever in this review; two individually randomized trials and five cluster randomized trials
- Results:
  - RDT supported diagnosis had little or no effect on the number of participants remaining unwell at four to seven days after treatment
- Results (cont'd)
  - Using RDTs to support diagnosis did not have a consistent effect on the prescription of antibiotics
- Algorithms incorporating RDTs can substantially reduce antimalarial prescribing if health workers adhere to the test results.
- Introducing RDTs has not been shown to improve health outcomes for patients, but adherence to the test result does not seem to result in worse clinical outcomes than presumptive treatment.

Odaga J et al. Rapid diagnostic tests versus clinical diagnosis for managing fever in settings where malaria is common. Cochrane Database of Systematic Reviews 2014, Issue 4. Art. No.: CD008998. DOI: 10.1002/14651858.CD008998.pub2.

CONCLUSIONS

- POCTs have the potential to improve the management of infectious diseases, especially in resource limited settings where health care infrastructure is weak, and access to quality and timely medical care is a challenge
- POCTs should fulfill the ASSURED criteria
  - A = Affordable
  - S = Sensitive
  - S = Specific
  - U = User-friendly (simple to perform in a few steps with minimal training)
  - R = Robust and rapid (can be stored at room temperature and results available
  - in <30 min)
  - E = Equipment-free or minimal equipment that can be solar-powered
  - D = Deliverable to those who need them



"If you want a second opinion, I'll ask my computer."

Thank you for your attention!