Point-of-care diagnostics and the role of rapid tests

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Greece
Definitions- Introduction to rapid tests

Methods used for rapid testing (RT)

RT for respiratory tract infections

RT for sexually transmitted infections

RT for bloodstream infections
What is POC?

- Point-of-Care testing is defined as various testing conducted near the site of patient care.
BEDSIDE TESTING DEVELOPMENT PROJECT
An Early Definition of POCT...

Tests done by non-laboratory staff outside a recognized diagnostic laboratory

This terminology replaces Near Patient Testing (NPT) as the favored term. Other terminologies include Bedside Testing, Extra-Laboratory Testing and Disseminated Laboratory Testing.

Today, POC incorporates different environments, including hospital based testing, near-patient testing, physician’s-office testing, and patient self testing.
Rapid Diagnosis of Infectious Diseases

- Accelerates the initiation of appropriate management
- May reduce unnecessary additional diagnostic testing and hospitalization
- Facilitates the prompt initiation of infection control policies
Attributes of Good Rapid Diagnostic Tests

- High sensitivity and specificity
- High – and + predictive value
- High accuracy relative to diagnostic gold standard
- Rapid turnaround time
- Simplicity
- Cost effectiveness
Are the test results clearly written and easy to understand by a non-microbiologist?
Results in minutes to 1-2 hours

Accurate, simple to use, low cost, easy to interpret, stable under extreme conditions, little or no processing, culturally acceptable.

Include “point of care” (for doctor) and “walk away” tests (home tests).

(Source http://www.rapid-diagnostics.org)
Rapid Diagnostic tests

...Rapid and accurate results on day 1:

- Supports decisions for appropriate and targeted therapy 1-3 days earlier than conventional methods
Methods

Antigen detection
Antibody detection
Molecular detection
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
Step 1: Add specimen

Step 2: Add agglutination solution

Step 3: Agitate sample, let settle, and observe for agglutination.
Latex agglutination test

Latex beads (= polystyrene particles)

bacterial Ag

Antibodies specific to Bacterial polysaccharide Ag

Source: WHO meningitis workshop Ouagadougou Sept 2004
Immunochromatography

- Lateral flow are also called immunochromatographic strip tests (ICS) or simply strip-tests.
- Specific qualitative or semi-quantitative detection of many analytes, including antigens, antibodies and even the products of nucleic acid amplification tests.
- Urine, saliva, serum, plasma, whole blood, feces, exudates can all be used as specimens.
A sample is placed on the sample pad at one end of the strip. The sample may be used alone as is commonly done with urine or serum compatible tests, or it may be mixed with a buffer specific to the test. This buffer may simply be a diluent/running buffer or it may be much more complex and have specific components or properties required to make the strip perform properly, such as a cell lyses buffer.
With the addition of the sample, the detector molecules are solubilized. When solubilized the detector molecules mix with and bind to the analyte in the sample (if analyte is present).

Then capillary action draws the fluid mixture up the sample pad and into the membrane. The sample/detector molecule mix continues to move up the membrane until it reaches the analyte capture molecule. In these lines a second (and third) antibody or antigen, immobilized as a thin stripe in the nitrocellulose will then capture the complex if it is positive for the target analyte. The control line should always show as a visible line, otherwise the test is invalid and must be repeated. If the test is positive, a colored (typically pink or purple) line develops along with the control line.
Excess buffer along with any reagents not captured at the test of control line will then move into the absorbent wicking pad.
Malaria P.f. RDT Results

NEGATIVE RESULTS
Wait 15 minutes before reading results.

POSITIVE RESULTS

INVALID RESULTS *
* No Control Lines (repeat tests)
Antibody 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
Molecular detection

Real-time PCR?

• DNA extraction < 1 h
• Simultaneous amplification and detection < 2 h
• Cost +++
Commercialized tests

<table>
<thead>
<tr>
<th>Family – genus – species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
</tr>
<tr>
<td><em>Clostridium perfringens</em>, <em>Corynebacterium diphteriae</em>, <em>Leptospira interrogans</em></td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em>, <em>Legionella pneumophila</em>, <em>Neisseria meningitidis</em></td>
</tr>
<tr>
<td><em>Salmonella Typhi</em>, <em>Yersinia pestis</em>, <em>Vibrio cholerae</em></td>
</tr>
<tr>
<td><strong>Virus</strong></td>
</tr>
<tr>
<td><em>Adenovirus</em>, <em>Rotavirus</em>, <em>Influenza virus</em>, <em>Dengue virus</em>, <em>SRV</em></td>
</tr>
<tr>
<td><strong>parasites</strong></td>
</tr>
<tr>
<td><em>Plasmodium sp</em>, <em>Giardia lamblia</em>, <em>Cryptosporidium</em></td>
</tr>
</tbody>
</table>

→ Not comprehensive
Advantages

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

Source: PATH RDT website: http://www.rapid-diagnostics.org
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

Source http://www.rapid-diagnostics.org
RDT use in algorithms

Algorithms are decision trees or visual schemes

Include behavioural, biological, or genetic risk factors for a disease, clinical signs and symptoms, use of other tests

Consider disease incidence and prevalence, availability and accuracy of other tests, probable consequences of misdiagnosis

Algorithms may be population specific and need to be updated periodically

Source: http://www.rapid-diagnostics.org
Cost

Cost analysis: cost of using this diagnostic test or algorithm in terms of personnel, facilities, equipment, sample collection materials, reagents, etc.

Cost-benefit analysis: cost of a correct diagnosis minus the benefits from reaching that correct diagnosis

- Averted treatment costs and losses due to illness (e.g. wages)
- Less tangible costs e.g. reduced pain and suffering to patients
- Very difficult to cost benefits accurately and there is much debate on the accuracy of cost-benefit analyses.

Source: http://www.rapid-diagnostics.org
Cost-efficience analysis: costs of a diagnostic test or algorithm compared to the health outcome resulting from the diagnosis.

Answers the following:
Relative to other tests or algorithms, is this test or algorithm a good use of health care funds?

Source: http://www.rapid-diagnostics.org
# Accuracy

## Positive and negative predictive values* for various HIV prevalences

<table>
<thead>
<tr>
<th>HIV prevalence</th>
<th>0.1%</th>
<th>1%</th>
<th>5%</th>
<th>10%</th>
<th>30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPV with one non-reactive test</td>
<td>100%</td>
<td>100%</td>
<td>99.9%</td>
<td>99.9%</td>
<td>99.9%</td>
</tr>
<tr>
<td>PPV with one reactive test</td>
<td>9%</td>
<td>50%</td>
<td>83.9%</td>
<td>91.7%</td>
<td>98.5%</td>
</tr>
<tr>
<td>PPV with two reactive tests</td>
<td>90.8%</td>
<td>99.0%</td>
<td>99%</td>
<td>99.9%</td>
<td>100%</td>
</tr>
</tbody>
</table>

*A sensitivity of 99% and a specificity of 99% have been used in these calculations. Predictive values have been rounded to one decimal place.

NPV = negative predictive value  
PPV = positive predictive value

Source: WHO Rapid HIV tests: guidelines for use in HIV testing and counselling services in resource-constrained settings, 2004
Barriers to use of RDT

Acceptability

• To policymakers, clinicians, and patients
• Sufficient sensitivity and specificity for the approval of international health and donor agencies
• Adequate predictive value and ease-of-use for clinicians, require culturally appropriate specimens
• Perceived as credible, to be accepted by patients
• Users must trust and accept RDT results, if tests are perceived as too simple, results may not be trusted

Source: http://www.rapid-diagnostics.org
Barriers to use of RDT

Affordability

- Many RDTs are more expensive than other tests or algorithms they are intended to replace
  - Especially true if RDT is used as a replacement for a syndromic algorithm
- Affordability constraints can be reduced by
  - Working to decrease the cost per test
  - Carefully designing algorithms to use the tests cost-effectively
  - Educating users of cost-savings for more efficient use of therapeutic drugs

Source: http://www.rapid-diagnostics.org
Barriers to use of RDT

Availability

• RDT not consistently available in many developing countries
• Most tests have a limited shelf life and many countries have poorly developed procurement and distribution systems
• The consistency and quality of imported tests
  – Local government regulations, quality assurance, shelf life testing, and distribution systems all need to be assessed and improved

Source: http://www.rapid-diagnostics.org
Role of laboratories

Central public health laboratories
• Developing algorithms
• Writing standard operating procedure (SOP)
• National External Quality Control Scheme organization
• RDT accuracy testing – a certification process
• Training courses at peripheral level
Role of laboratories

Peripheral laboratories need:
• Well trained people
• SOP and quality assurance culture
• Basic laboratory equipment (refrigerator, pipettes and tips, water bath)
• Sample collection and transportation experience
• Data collection and registration experience
→ Try to involve lab specialist as much as possible
Conclusion

- RDT should be used in outbreak detection and investigation
- Several manufacturers
- Be aware of the limitations and constraints
- Use algorithms
- Involve laboratories at central and peripheral level
Additional resources

Medecins Sans Fontieres (video on meningitis RDT)

Malaria RDT http://www.wpro.who.int/rdt/
  • guidelines, reviews, trials etc.

http://www.rapid-diagnostics.org/
RTs for respiratory tract infections
Rapid streptococcal antigen tests (RSATs) are based upon enzyme or acid extraction of antigen from throat swabs.

Most tests currently in use employ enzyme linked immunosorbent assay (ELISA) to detect antigen-antibody complexes.

Sensitivity for RSAT ranges from 70 to 90 percent and specificity from 90 to 100 percent in multiple studies.
The performance of a particular RSAT for the diagnosis of GAS pharyngitis is also a function of the clinical characteristics of the illness in patients selected for testing. This factor is referred to as "spectrum bias".

In three studies, the sensitivity of the RSAT increased concomitant with the number of Centor criteria present.
Strep test

- A positive RSAT is useful in establishing the diagnosis of GAS pharyngitis, but a negative test does not rule out GAS.
- The RSAT provides a same visit result and permits rapid institution of therapy.
- It does not identify non-group A beta hemolytic streptococci.
Pneumonia in an adult

A 66 yr old medical school professor was traveling in India, Hong Kong, Singapore, and Bangkok when he developed hoarseness, followed over the next 4 days by bronchospasm, non-productive cough, fever and fatigue.
Pneumonia in an adult

He treated himself with bronchodilators, tylenol, and then a tapering course of steroids.

He terminated his trip and returned to the U.S., however, he required a wheelchair to change planes.

Past medical history: Asthma for > 60 years.
Pneumonia in an adult

On return home, the professor went to the Emergency Department.

Physical exam revealed T = 99.7°F, RR = 22, scattered wheezes, and rales in left lower lung field.

paO2 was 89% and chest x-ray revealed pneumonia in the left lower lobe and lingula.

WBC 12,100
What do you think is the most likely viral diagnosis?

1. Influenza
2. Avian Influenza
3. SARS
4. RSV
5. Parainfluenza
6. Adenovirus
What rapid viral diagnostic test does your laboratory routinely perform on an ADULT with pneumonia?

1. Rapid influenza test
2. Rapid RSV test
3. Both influenza and RSV
4. Rapid respiratory screen (e.g. RSV, influenza, parainfluenza, adenovirus)
5. None of the above
What is the main rapid test METHOD you perform for respiratory viruses?

1. EIA
2. OIA, Zstat Flu, Binax or other newer test
3. Immunofluorescence
4. Shell vial culture
5. RT-PCR or other molecular method
6. No rapid tests, only culture
Pneumonia in an adult

- The patient was producing no sputum.
- A nasopharyngeal swab was sent for a viral diagnostic test.
Are samples for rapid respiratory testing batched in your laboratory?

1. Yes
2. No
What is your average turnaround time during operating hours?

1. 30 minutes or less
2. One hour
3. Two hours
4. Three hours
5. Four hours
6. More than 4 hours
Pneumonia in an adult

- Respiratory Screen DFA was strongly positive for Respiratory syncytial virus

Result reported to ED prior to admission
RSV-infected respiratory epithelial cells (IF-rhodamine label)
RSV Pneumonia in an adult

- The patient was treated with oxygen, respiratory therapy with bronchodilators, and steroids.
- After 5 days in the hospital, he was discharged to home.
- 5 days later, he was back at work part time, and by 7 days was at work full time.
RSV Pneumonia in Adults

- First reported in the 1960s
- Increased risk for serious disease:
  - Adults with underlying cardiopulmonary disease
  - Frail elderly persons
  - The severely immunocompromised
Clues to RSV in the Elderly

- Typically begins with nasal congestion and discharge
- Cough affects 90-97%
- Fever in 50%, typically lower than flu
- Rales and wheezes on exam in 30-40%
- Pneumonia often alveolar, but may be interstitial; lobar consolidation in 35%
Diagnosis in cases of Atypical Pneumonias

- By serological methods using acute and convalescent sera
- Raise of significant titer or rising titer of antibodies give clues to diagnosis.
Newer methods - Diagnosis of Community associated Pneumonia

- Antigen detection in sputum or urine by Fluorescent methods
- Immunoelectrophoresis
- Latex agglutination tests
- ELISA
Emerging methods in Diagnosis

- Newer amplified DNA detection methods likely to improve the diagnosis of several cases of pneumonias.
Advances in Diagnosis of CAP

- Polymerase chain reaction assay for *Mycoplasma pneumoniae* from throat swab or sputum culture and urinary or serologic antigen tests for *Legionella* have made inroads into accurately diagnosing CAP caused by atypical organisms.

- Urinary antigen detection of the C polysaccharide common to *Streptococcus pneumoniae* now rapidly identifies the presence of this organism. To obtain higher yield specimens, transthoracic needle aspiration and bronchoscopic protected specimen brush techniques are being used among sicker patients.
## Community-acquired pneumonia

<table>
<thead>
<tr>
<th>Indication</th>
<th>Blood culture</th>
<th>Sputum culture</th>
<th>Legionella UAT</th>
<th>Pneumococcal UAT</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICU admission</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>Aspirate</td>
</tr>
<tr>
<td>Failure of outpatient antibiotic therapy</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cavitary infiltrates</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td>Fungi/TB</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active alcohol abuse</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic liver disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe obstructive/structural lung disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asplenia</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recent travel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive L. UAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive P. UAT</td>
<td>x</td>
<td></td>
<td></td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td>Thoracentesis</td>
</tr>
</tbody>
</table>
## Diagnostic tests for Legionella species

<table>
<thead>
<tr>
<th>Test characteristic</th>
<th>Culture</th>
<th>Urine antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strains detected</td>
<td>All species and serogroups</td>
<td>Detects only L. pneumophila, serogroup 1; this accounts for 70 to 80 percent of cases</td>
</tr>
<tr>
<td>Speed to results</td>
<td>≥3 days</td>
<td>15 minutes with some assays</td>
</tr>
<tr>
<td>Technical demands</td>
<td>Difficult - high rates of false negatives</td>
<td>Easy - Rare false negatives except with strains other than L. pneumophila, serogroup 1</td>
</tr>
<tr>
<td>Overall sensitivity</td>
<td>&lt;10 to 80 percent</td>
<td>70 to 80 percent*</td>
</tr>
<tr>
<td>Overall specificity</td>
<td>100 percent</td>
<td>&gt;99 percent</td>
</tr>
</tbody>
</table>

Hamman-Rich syndrome revisited: how to avoid misdiagnosis

Jiro Fujita, a Masato Tohyama, b Shusaku Haranaga, a Haley L. Cash, b Futoshi Higa, a Masao Tateyama a

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Case report

- 59-year-old man, obese
- Fever, sore throat
- Rapid test for influenza (swab): negative
- Symptoms continued. Shortness of breath, crackles bilaterally.
- Second rapid test for influenza (swab): negative
Question

Which statement is correct:

1. The patient does not have influenza
2. Rapid antigen tests for influenza have high sensitivity and low specificity
3. Rapid antigen tests for influenza have low sensitivity and high specificity
4. Rapid antigen tests for influenza have high sensitivity and specificity
Question

Which statement is correct:

1. The patient does not have influenza
2. Rapid antigen tests for influenza have high sensitivity and low specificity
3. Rapid antigen tests for influenza have low sensitivity and high specificity
4. Rapid antigen tests for influenza have high sensitivity and specificity
Chest x-ray and CT-scan: Ground-glass and infiltrative shadow in both lung fields.
Bronchoalveolar lavage

Transbronchial biopsy: Diffuse alveolar damage compatible with acute interstitial pneumonia.

BAL fluid:
- PCR for pandemic H1N1 2009 POSITIVE
- Rapid test for influenza A POSITIVE.

If PCR for pandemic H1N1 2009 had not been performed, the patient would have been diagnosed with acute interstitial pneumonia: Hamman-Rich syndrome.
Laboratory diagnosis of influenza

- Reverse transcriptase PCR: the most sensitive and specific
- Rapid antigen tests
- Immunofluorescence testing

Useful screening tests, but have limited sensitivity.
Rapid influenza antigen tests

- Immunoassays that can identify influenza A and B viral nucleoprotein antigens in respiratory specimens

- In a meta-analysis of 159 studies that evaluated rapid influenza antigen tests, the pooled sensitivity was 62.3 percent (95% CI 57.9-66.6 percent) and the pooled specificity was 98.2 percent (95% CI 97.5-98.7 percent). The sensitivity was lower in adults than in children (53.9 versus 66.6 percent), and was higher for influenza A than for influenza B (64.6 versus 52.2 percent).

Rapid influenza antigen tests

- Viral shedding peaks at 24 to 48 hours of illness and then rapidly declines; little or no detectable viral replication occurs in the respiratory tract after 5 to 10 days in immunocompetent hosts.

- Longer periods of shedding can occur in children, elderly adults, patients with chronic illnesses, and immunocompromised hosts.

- The live attenuated influenza vaccine can cause a false positive result on a rapid influenza diagnostic test, since these tests cannot differentiate between live attenuated and wild-type influenza viruses.
# Influenza testing methods

<table>
<thead>
<tr>
<th>Test</th>
<th>Time to results</th>
<th>Acceptable specimens</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct fluorescent antibody staining</td>
<td>2 h</td>
<td>NP swab, NP or bronchial washing, nasal or endotracheal aspirate, sputum, throat swab*</td>
<td>High sensitivity and very high specificity; highly recommended; can differentiate between influenza types (A or B) and subtypes (including pandemic H1N1 influenza and avian H5N1 influenza)</td>
</tr>
<tr>
<td>Indirect fluorescent antibody staining</td>
<td>2 h</td>
<td>NP swab, NP or bronchial washing, nasal or endotracheal aspirate, sputum, throat swab*</td>
<td></td>
</tr>
<tr>
<td>Rapid influenza diagnostic testsΔ</td>
<td>10-20 min</td>
<td>NP swab, nasal washing, nasal aspirate, throat swab*</td>
<td>Low sensitivity, high specificity; detection time dependent on assay, and A and B, or both can be detected</td>
</tr>
<tr>
<td>Antigen detection (EIA)</td>
<td>20-30 min</td>
<td>NP swab, nasal washing, nasal aspirate, throat swab*</td>
<td>Detects influenza, not highly specific</td>
</tr>
<tr>
<td>Neuraminidase detection assay</td>
<td>20-30 min</td>
<td>NP swab, nasal washing, nasal aspirate, throat swab*</td>
<td></td>
</tr>
<tr>
<td>Viral culture</td>
<td>48-72 h</td>
<td>NP swab, nasal washing, nasal aspirate, sputum, throat swab*</td>
<td>Requires paired acute- and convalescent-phase serum samples, isolation in cell culture, and serologic tests (hemagglutinin inhibition, ELISA, complement-fixation, and neutralization)◊</td>
</tr>
<tr>
<td>Shell viral culture</td>
<td>5-10 days</td>
<td>NP swab, nasal washing, nasal aspirate, sputum, throat swab*</td>
<td></td>
</tr>
<tr>
<td>Serologic tests (hemagglutinin inhibition, ELISA, complement-fixation, and neutralization)◊</td>
<td>Serum</td>
<td>Available only in reference laboratories; not useful for timely clinical management; recommended only for retrospective diagnosis, surveillance, or research purposes</td>
<td></td>
</tr>
</tbody>
</table>

RT-PCR: reverse-transcriptase polymerase chain reaction; NP: nasopharyngeal; EIA: enzyme immunoassay; ELISA: enzyme-linked immunosorbent assay.

* Throat swabs are inferior to NP specimens for the detection of influenza viruses, but may be used if NP specimens cannot be obtained.

Δ Includes moderately complex and Clinical Laboratory Improvement Amendments (CLIA)-waived tests.

◊ Requires paired acute- and convalescent-phase serum samples.

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<tr>
<td>RT-PCR (conventional gel-based PCR, real-time RT-PCR, and multiplex PCR)</td>
<td>2 h</td>
<td>NP swab, NP or bronchial washing, nasal or endotracheal aspirate, sputum, throat swab*</td>
<td>High sensitivity and very high specificity; highly recommended; can differentiate between influenza types (A or B) and subtypes (including pandemic H1N1 influenza and avian H5N1 influenza)</td>
</tr>
<tr>
<td>Immunofluorescence direct or indirect antibody staining</td>
<td>2-4 h</td>
<td>NP swab or washing, bronchial washing, nasal or endotracheal aspirate</td>
<td>Moderately high sensitivity and high specificity; recommended</td>
</tr>
<tr>
<td>Antigen detection (EIA)</td>
<td>10-20 min</td>
<td>...</td>
<td>Depends either on A or B, or both</td>
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<td>Neuraminidase detection assay</td>
<td>20-30 min</td>
<td>...</td>
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*Throat swabs are inferior to NP specimens for the detection of influenza viruses, but may be used if NP specimens cannot be obtained.
*Requires fluorescence microscope.
△Includes moderately complex and Clinical Laboratory Improvement Amendments (CLIA)-waived tests.
*Requires paired acute- and convalescent-phase serum samples.
### Rapid influenza diagnostic tests
- **Antigen detection (EIA)**
- **Neuraminidase detection assay**

#### Influenza testing methods

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<td>High sensitivity and very high</td>
</tr>
<tr>
<td>real-time RT-PCR, and multiplex PCR)</td>
<td></td>
<td>aspirate, sputum, throat swab*</td>
<td>specificity; highly recommended; can differentiate between influenza</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>types (A or B) and subtypes (including pandemic H1N1 influenza and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>avian H5N1 influenza)</td>
</tr>
<tr>
<td>Immunofluorescence*</td>
<td>10-30 min</td>
<td>NP swab or washing, bronchial washing, nasal or</td>
<td>Low to moderate sensitivity and high</td>
</tr>
<tr>
<td></td>
<td></td>
<td>endotracheal aspirate, sputum, throat swab*</td>
<td>specificity; recommended; during periods of peak influenza activity,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>negative rapid antigen tests do not reliably exclude influenza</td>
</tr>
<tr>
<td>Neuraminidase detection assay</td>
<td>20-30 min</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Viral culture</td>
<td>48-72 h</td>
<td>NP swab, NP or bronchial washing, nasal or endotracheal</td>
<td>Mode higher sensitivity, important test for surveillance</td>
</tr>
<tr>
<td>Isolation in cell culture</td>
<td>5-10 days</td>
<td>aspirate, sputum, throat swab*</td>
<td></td>
</tr>
<tr>
<td>Serologic tests (Hemagglutinin inhibition, ELISA, complement fixation, and neutralization)</td>
<td>...</td>
<td>Serum</td>
<td>Available labor clinic only</td>
</tr>
</tbody>
</table>
Multiplex PCR and Emerging Technologies for the Detection of Respiratory Pathogens

Angela M. Caliendo
Department of Pathology and Laboratory Medicine, Emory University School of Medicine; Emory Center for AIDS Research, Emory University, Atlanta, Georgia

Clinical Infectious Diseases 2011;52(S4):S326–S330
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RespPlex</th>
<th>Infiniti</th>
<th>Jaguar</th>
<th>FilmArray</th>
<th>STAR</th>
<th>PLEX-ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogens detected</td>
<td>Viruses and bacteria</td>
<td>Viruses</td>
<td>Viruses</td>
<td>Viruses and bacteria</td>
<td>Viruses</td>
<td>Viruses and bacteria</td>
</tr>
<tr>
<td>Degree of multiplexity, no. of targets</td>
<td>&gt;15</td>
<td>&gt;15</td>
<td>&gt;6</td>
<td>&gt;15</td>
<td>&gt;15</td>
<td>&gt;15</td>
</tr>
<tr>
<td>Complexity</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Fully integrated system (all steps)</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Testing location</td>
<td>Laboratory</td>
<td>Laboratory</td>
<td>Near-patient facility and/or laboratory</td>
<td>Near-patient facility and/or laboratory</td>
<td>Laboratory</td>
<td>Laboratory</td>
</tr>
<tr>
<td>Time required for result, h</td>
<td>5-6</td>
<td>6.5-10</td>
<td>1.5-2</td>
<td>1</td>
<td>5-6</td>
<td>6-8</td>
</tr>
<tr>
<td>Throughput</td>
<td>Moderate to high</td>
<td>Moderate to high</td>
<td>Moderate</td>
<td>Low</td>
<td>Moderate to high</td>
<td>Moderate to high</td>
</tr>
<tr>
<td>Carryover contamination risk</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Quantification</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Pathogen discovery</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*These data reflect the state of technology as of October 2009; manufacturers may alter their test systems in the future.*
RTs for sexually-transmitted infections

A SERIOUS PROBLEM IN DEVELOPING COUNTRIES
The need for STI diagnostics

- The WHO estimates that more than 380 million new cases of sexually transmitted chlamydia, gonorrhea, syphilis and trichomoniasis occur worldwide every year.

- Undiagnosed and untreated STIs can lead to long term complications.

- The control of curable STIs in countries with high disease burden has been hampered by the lack of accessible STI laboratory services.

What is syndromic management?

- Evaluations of the WHO flowcharts have shown that the algorithm for vaginal discharge lacks both sensitivity and specificity for the identification of women with *C. trachomatis* and *N. gonorrhoeae* infection.

- In some areas of the world, syndromic management can result in as much as 98% overtreatment for chlamydia and gonorrhoea in women presenting with vaginal discharge.
The ideal rapid test for STIs: ASSURED criteria

A = Affordable
S = Sensitive
S = Specific
U = User friendly (simple to perform in a few steps)
R = Robust and rapid (thermostable and results in <30min)
E = Equipment free
D = Deliverable to those who need them
Syphilis

- Syphilis is a sexually transmitted disease caused by the spirochete Treponema pallidum.
- The RPR (Rapid Plasma Reagin) Card test is a presumptive serologic screening test for syphilis.
- The serum of a person with syphilis contains a non-specific anti-lipid antibody (termed reagin), which is not found in normal serum.
Syphilis

- Syphilis infection starts the breakdown of the patient's own tissue cells.
- Fatty substances which are released combine with protein from Treponema pallidum to form an antigen which stimulates the body to produce antibodies against both the body's tissue lipids (non-specific or non-treponemal) as well as the T. pallidum protein (specific or treponemal).
- The RPR Card test detects the nonspecific antilipid antibody and is referred to as a non-treponemal test for syphilis.
Syphilis

- Syphilis in pregnancy is a major cause of adverse pregnancy outcome.

- In most countries non-treponemal tests such as the Rapid Plasma Reagin (RPR) or the Venereal Diseases Research Laboratory (VDRL) slide test is used to screen pregnant women.

- Women who test positive at peripheral clinics are treated without confirmatory tests as treponemal tests are not widely available.
A pregnant woman has a positive RPR/VDRL test. Which of the following statements is correct:

1. She may have syphilis
2. She may have malaria
3. She may have leprosy
4. She may have an immune disorder
5. All of the above
Question

A pregnant woman has a positive RPR/VDRL test. Which of the following statements is correct:

1. She may have syphilis
2. She may have malaria
3. She may have leprosy
4. She may have an immune disorder
5. All of the above
Rapid tests for syphilis

- Where laboratory services are not available, the new simple, point-of-care treponemal tests, in a dipstick or cassette format, provide an important opportunity to improve access to testing.

- Some rapid syphilis tests have sensitivities of 85-99% and specificities of 93-100% compared to laboratory based treponemal tests.
Rapid tests for syphilis

- These tests do not require equipment, can be transported and stored at room temperature and can be used with whole blood obtained by finger pricks, although sensitivity can decrease by 10-20%.

- Rapid tests, unlike RPR or VDRL, do not give false negative results with specimens containing high level of antibodies, a phenomenon known as the prozone effect.
Rapid tests for syphilis

- However, a disadvantage of these rapid treponemal tests is that they cannot be used to distinguish between recent active infection and past treated infection, as treponemal antibodies persist for years.

- But given the serious consequences of failure to detect and treat infected pregnant women and the rarity of adverse drug effects, the benefits of a rapid treponemal test that is simple to perform and enable immediate treatment clearly outweigh the risks of over-treatment.
Chlamydia and gonorrhea

- Four diagnostic companies have marketed nucleic acid amplified tests (NAATs) for the diagnosis and screening of genital chlamydial and gonococcal infections. These tests can detect 10-100 bacteria and have specificities greater than 98%.

Chlamydia and gonorrhea

- With the sensitivity of NAATs, it is now possible to use non-invasive specimens such as urine and self- or physician collected vaginal swabs instead of urethral or cervical swabs.

- However, these tests require sophisticated equipment, highly trained personnel and are costly. They are not widely available in most of the developing world where the disease burden of bacterial STIs is greatest.
Rapid tests for chlamydia and gonorrhea

- More than 20 on the market.
- Recent evaluations showed that most of them have adequate specificity but with the exception of one test that has a sensitivity of more than 80% for vaginal swabs compared to urine PCR in women, most have sub-optimal sensitivity of approximately 50%-70% compared to PCR for cervical swabs and 33%-70% for vaginal swabs.
Question

Which method results in a larger number of women being treated for chlamydia:

1. NAAT with a sensitivity of 90%
2. Rapid test with a sensitivity of 65%
Question

Which method results in a larger number of women being treated for chlamydia:

1. NAAT with a sensitivity of 90%

2. Rapid test with a sensitivity of 65%!!!
Rapid Test Paradox

- Using a decision analysis in a US STD clinic setting, Gift et al. showed that screening for chlamydia in women using a rapid chlamydia test with 65% sensitivity can result in more cases of chlamydia being treated compared to a NAAT with 90% sensitivity, since NAATs have a much longer turn around time and many patients failed to return for their test results.

STIs in developing countries

- Rapid tests for STIs have still many problems.
- With more political commitment and technological advances made possible by increasing number of funders and product development partnerships, there is much optimism in the near future for point of care tests for STIs that can improve patient management and disease control.
Rapid test for HIV
What is the Rapid Test?

- HIV antibody test
- Screening test
- Fingerstick blood/Oral specimen
- Preliminary results in 20 minutes
- Non-reactive results do not require further testing
- Reactive results must be confirmed
What’s Different?

**Standard Testing**
- Draw specimen intravenously
- Send specimen to lab
- Lab conducts lab QA
- Lab performs screen (ELISA)
- If reactive, lab performs confirmatory (Western Blot)
- Lab sends results
- Second session: All results to client

**Rapid Testing**
- Conduct lab QA
- Draw specimen by fingerstick or collect oral fluid
- Perform screen
- Give results to client
- If reactive, collect specimen (Intravenous blood sample)
- Send specimen to lab
- Lab performs confirmatory
- Lab sends results
- Second session: confirmatory results to client
Rapid HIV Test

- Requires minimal training
- Point-of-care
- CLIA waived
- High-client satisfaction
- Accurate
- Less potential for clients lost to follow-up
### Performance Characteristics:
**OraQuick® Advance Rapid HIV-1/2 Antibody Test**

<table>
<thead>
<tr>
<th></th>
<th>Fingerstick</th>
<th>Oral</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV 1</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>99.6%</td>
<td>99.3%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>99.8%</td>
</tr>
</tbody>
</table>

* Limited Data for HIV-2. See Test Kit Insert
How Accurate Is It?

Predictive Value

The probability that the test result predicts the true infection status of the person tested.

Does this test result correctly reflect the disease state of the person tested?

- **High Negative Predictive Value**
  - Non-reactive = Client was uninfected 3 months ago

- **Variable Positive Predictive Value**
Positive Predictive Value

Varies depending on prevalence of infection in the population being tested.

Low prevalence = low positive predictive value.
- 0.1% prevalence = as low as 33% positive predictive value.
- 2 out of 3 reactive results are likely to be false positives.

High prevalence = high positive predictive value.
- 10% prevalence = can be 98% positive predictive value.
- Only 2 out of 100 reactive results are likely to be false positives.
OraQuick® Advance Rapid HIV-1/2 Antibody Test Kit
Test Device
Fingerstick Specimen

Fingerstick blood specimen & specimen collection loop
Blood-filled specimen collection loop
Insert specimen collection loop into developer solution vial and stir
Insert test device into developer solution vial, stir.
Read test in 20-40 minutes
Oral Fluid Specimen
Test develops in 20 - 40 minutes
Test Procedure

1. Open Pouch
2. Place open developer vial in the stand
3. Collect and mix sample in developer
4. Put test device in the developer vial
5. Let the reaction occur for 20 min.
6. Read results after 20 min & no later than 40 min
Rapid Test Results

- Reactive
- Non-Reactive
- Invalid
Reading Results

Reactive Specimen

Reactive Control

Reactive

Non- Reactive
Reading Results (2)

Invalid Test Results
Non-Reactive results require no further testing at this time

Uninfected as of 3 months ago
Reactive results must be confirmed!
### Properties of rapid HIV tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimens</th>
<th>CLIA status</th>
<th>Time (min)</th>
<th>HIV 1 + 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>OraQuick</td>
<td>Blood, plasma oral fluid</td>
<td>Waived</td>
<td>20</td>
<td>HIV 1 &amp; 2</td>
</tr>
<tr>
<td>UniGold</td>
<td>Blood/plasma/serum</td>
<td>Waived</td>
<td>10</td>
<td>HIV-1</td>
</tr>
<tr>
<td>Reveal G2</td>
<td>Plasma/serum</td>
<td>Mod complex</td>
<td>5</td>
<td>HIV-1</td>
</tr>
<tr>
<td>Multispot</td>
<td>Serum</td>
<td>Mod complex</td>
<td>15</td>
<td>HIV-1 &amp; 2</td>
</tr>
<tr>
<td>Clearview</td>
<td>Blood/serum</td>
<td>Waived</td>
<td>15-20</td>
<td>HIV-1 &amp; 2</td>
</tr>
<tr>
<td>VITROS</td>
<td>Serum</td>
<td>Mod complex</td>
<td>50</td>
<td>HIV-1 &amp; 2</td>
</tr>
</tbody>
</table>

*Courtesy of John G. Bartlett, MD.*
RTs for bloodstream infections
Bench-to-bedside review: Rapid molecular diagnostics for bloodstream infection - a new frontier?

Arash Afshari¹², Jacques Schrenzel³, Margareta Ieven⁴ and Stephan Harbarth¹*
### Table 2. Current molecular techniques for detection of bloodstream infection, performed directly on whole blood

<table>
<thead>
<tr>
<th>Assay/manufacturer</th>
<th>Technique</th>
<th>Anti-microbial resistance genes</th>
<th>Detectable pathogens</th>
<th>Detection limit (CFU/ml)</th>
<th>Turn-around time (h)</th>
<th>Costs equipment/supplies</th>
<th>Sensitivity/specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SepsTest (Malzyn, Germany)</td>
<td>Broad-range PCR + sequencing</td>
<td>None</td>
<td>&gt;300 different pathogens</td>
<td>20-40 for S. aureus</td>
<td>8-12</td>
<td>$$$/+ + + +</td>
<td>82%/86-90%</td>
</tr>
</tbody>
</table>
| Vyo (SIRS-Lab, Germany) | Multiplex PCR + gel electrophoresis | mec, vanA, vanB, vanC, bla
| Plex-ID (Abbott, USA) | Multiplex PCR detected by electrospray ionization mass spectrometry | mecA, vanA, vanB, vanC, bla | Multiple pathogens and biogents, including Yersinia pestis, Bacillus anthracis. Separate dedicated assays for viruses or yeasts/fungi | NA                       | 8-12                 | $$$$/+ + + +               | NA/NA                         |
| VACPchip [106] (Eppendorf Array Technologies, Rottach-Egern) | Multiplex PCR + hybridization on a cartridge-containing microarray | bla
| bla
| bla | S. aureus, S. pneumoniae, E. coli, K. pneumoniae, K. oxytoca, E. cloacae, E. aerogenes, S. marcescens, A. baumannii, P. aeruginosa, C. albicans | >10                      | 5-8                  | NA                        | NA/NA                      |
These organisms are responsible for > 90% of bacteremias

**Gram (-)**
- Escherichia coli
- Klebsiella (pneumoniae / oxytoca)
- Serratia marcescens
- Enterobacter (cloacae / aerogenes)
- Proteus mirabilis
- Pseudomonas aeruginosa
- Acinetobacter baumannii
- Stenotrophomonas maltophilia

**Gram (+)**
- Staphylococcus aureus
- CoNS (Coagulase negative Staphylococci)*
- Streptococcus pneumoniae
- Streptococcus spp.**
- Enterococcus faecium
- Enterococcus faecalis

**Fungi**
- Candida albicans
- Candida tropicalis
- Candida parapsilosis
- Candida krusei
- Candida glabrata
- Aspergillus fumigatus
Rapid identification of species with LightCycler® SeptiFast Test MGRADE
Conclusions

- Point-of-care diagnostics are changing the practice of medicine.
- There are still many obstacles to surpass.
- However, there are many advances in the field, which give us the hope that we will be able to treat our patients faster and more efficiently.
Thank you!