The Application of Molecular POCT for Influenza and Strep Detection
Challenges and Solutions

Gregory J. Berry, Ph.D., D(ABMM)
Assistant Professor, Pathology and Laboratory Medicine
Zucker School of Medicine at Hofstra/Northwell
Director of Molecular Diagnostics and Microbiology
Northwell Health Labs and Long Island Jewish Medical Center
Assistant Director, Infectious Disease Diagnostics
Disclosures

• Provided educational talks and received honoraria from Abbott, BioFire, Cardinal health, Cepheid, Hologic, Luminex, Quidel
Objectives

• Introduce Point-of-Care Testing (POCT) for infectious diseases

• Review different molecular testing methodologies

• Focus on CLIA-waived molecular testing options for infectious diseases

• Review data from a Group A strep POC study
Testing for pathogens:

**Testing in Clinic (Point of Care)**
- Rapid antigen tests
- **Molecular nucleic acid amplification testing (POCT)**

**Laboratory Testing**
- Culture workup (e.g. Strep)
- Molecular nucleic acid amplification
Point-of-care testing (POCT)

- Testing performed while patient care is occurring
- Main advantage is time gained
- Therapeutic choices in real time
  - Identify treatment to administer
  - Avoid unnecessary drugs/treatments
- Requires simple platforms with accurate results

https://i.pinimg.com/736x/0c/26/a9/0c26a969cd5be705139c9a71f39e3665--point-of-care-testing-lab-tech.jpg
Barriers to POCT and Solutions

**Problems**
- Not accurate enough for definitive diagnosis
  - E.g. rapid strep and flu tests
- Too difficult to perform at point-of-care
  - E.g. molecular testing
- Too Expensive

**Solutions**
- Increasing sensitivity and specificity
- Assays designed to be user-friendly and more error-proof
- Costs decreasing over time and reimbursement that matches test costs
POCT in Infectious Disease Diagnostics

• CLIA waived tests that can be performed by facilities with a Certificate of Waiver

• Increasingly larger portion of infectious disease testing

• **Huge advantage of rapid answer for treatment decisions**

• QUALITY is key- results must approach the same sensitivity and specificity of laboratory tests
Timing is Everything!

- Patients Most Infectious during first 3-5 days\(^1\)
- Antiviral Drugs Most Effective during first 2-4 days\(^{1,2}\)
- Rapid POCT Sensitivity Highest\(^3\)

Days Post Symptom Onset:

- 0: High Viral Titer
- 1-3: Patients Most Infectious
- 4-10: Low Viral Titer

1. Reference: [1]
2. Reference: [2]
3. Reference: [3]
So is Specimen Collection!

The right specimen for the right test is key!
Types of POCTs available for Infectious Diseases


• Two basic types of tests
  • Rapid antigen detection tests
    • Detecting host antibodies produced against pathogen
    • Directly detecting antigens of pathogen
  • Molecular assays
Rapid antigen tests

• Available since the 1980s - Flu, GAS, RSV

• Most common first line of testing at clinic

• Immunoassays: detect pathogen-specific antigens

• Qualitative resulting

• Vary greatly in their sensitivity
  • Negative GAS results need culture confirmation
Notes from the Field

Group A Streptococcal Pharyngitis Misdiagnoses at a Rural Urgent-Care Clinic — Wyoming, March 2015

Alexia Harrist, MD, PhD; Clayton Van Houten, MS; Stanford T. Shulman, MD; Chris Van Beneden, MD; Tracy Murphy, MD

Group A Streptococcus (GAS) is the most common bacterial cause of pharyngitis, implicated in 20%–30% of pediatric and 5%–15% of adult health care visits for sore throat (1). Along with the sudden onset of throat pain, GAS pharyngitis symptoms include fever, headache, and bilateral tender cervical lymphadenopathy (1,2). Accurate diagnosis and management of GAS pharyngitis is critical for limiting antibiotic overuse and preventing rheumatic fever (2), but distinguishing between GAS and viral pharyngitis clinically is challenging (1). Guidelines for diagnosis and management of GAS pharyngitis have been published by the Infectious Diseases Society of America (IDSA)* (1). IDSA recommends that patients with sore throat be tested for GAS to distinguish between GAS and viral pharyngitis; however, IDSA emphasizes the use of selective testing based on clinical symptoms and signs to avoid identifying GAS carriers rather than acute GAS infections (1). Therefore, testing for GAS usually is not recommended for the following: patients with sore throat and accompanying

The line list revealed nonadherence to IDSA guidelines in testing and treatment procedures. Ten of 34 (29%) patients aged ≥3 years who were tested for GAS reported no sore throat; the symptom that should prompt evaluation for GAS pharyngitis in patients aged ≥3 years (1). Two of these 10 were asymptomatic adult contacts of patients with diagnosed GAS pharyngitis; both asymptomatic contacts had positive RADT results and were prescribed an antibiotic. Of the 24 tested patients aged ≥3 years with sore throat, 19 (79%) reported cough or rhinorrhea, symptoms that suggest a viral rather than bacterial etiology (1). Although diagnostic testing of patients aged <3 years is not routinely recommended, testing of symptomatic children who are household contacts of persons with laboratory-confirmed GAS pharyngitis can be considered (1). Among the seven patients aged <3 years who were tested for GAS pharyngitis, five (71%) had GAS-positive family members indicated by shared surname included in the line list; however, all seven (100%) had cough, and five (71%) had rhinorrhea.

Four of six patients with negative RADT results received an antibiotic. The clinic practice was to send throat swabs from patients with negative RADTs to a commercial laboratory for back-up culture, but it is unknown whether the clinic obtained GAS-positive throat cultures from RADT-negative patients.
Investigation

• In March 2015, a rural urgent-care clinic serving a population of 5,000–7,000 reported a substantial increase in GAS pharyngitis infections since November 2014, with some infections nonresponsive to penicillin and amoxicillin to the Wyoming Department of Health (WDH).

• Findings:
  • Testing asymptomatic patients (no sore throat)
  • Testing of patients with viral illness symptoms
  • 86% positivity rate on rapid antigen tests performed
  • Clinic staff were reading tests at longer intervals than manufacturer’s instructions - can lead to false positives

• Intervention
  • Cases declined, no resistance was found - likely viral illnesses misdiagnosed as GAS

Follow the FDA approved package insert!!!
Laboratory testing

• Testing performed in centralized location

• Lab is licensed and accredited to perform patient testing

• Licensed laboratory personnel perform testing

Identification of GAS by culture

• Throat swab is collected and inoculated onto plates
  • Most laboratories are routinely identifying only GAS
    • Agar selective for strep, or BAP can be used with a Bacitracin (A) disk
      • GAS is SUSCEPTIBLE
    • Additional workup can be done if other pathogens suspected
      • e.g. blood agar for other streptococci (B, C, F, G) and A. haemolyticum, or modified Thayer-Martin for N. gonorrhoeae isolation

• Incubate in aerobic incubator with 5% CO₂

• Result: 24-48 hours
Molecular testing

- In general, molecular methods are the most sensitive/specific testing

- Advantage for clinician - rapid turnaround time (hours, even minutes vs. 1-3 days)

- Downstream impact:
  - More rapid answer means *right therapeutic therapy* chosen up-front
  - Patient satisfaction
Traditional Molecular Laboratory testing platforms

• Highly sensitive/specific

• Performed in the laboratory by specialized personnel
  • **NOT POC-friendly**

• TAT can be 1-2 hours (*once received*, if NOT batched)
  • If batched, could be 12-24 hours, depending on how often testing is performed
The quality and power of sample amplification

Detection threshold

Amplified Sample

Not Amplified Sample

Nurse A

Nurse B

Sample

Sample

Amplified

Not Amplified
CLIA-waived Molecular POCT options
ID NOW™ (Abbott, formerly Alere™ i)

- 5-13 minutes to result for Flu
- ≤13 minutes to result for RSV
- 2-6 minutes to result for Strep A
- Isothermal Amplification
- Interpreted by instrument

Flu: CLIA-waived for use with nasal or nasopharyngeal swabs (direct and eluted in viral transport medium)

For in vitro Diagnostic Use
ID NOW™ Strep A 2 clinical trial data, held on file
Cepheid Xpert® Xpress Flu/RSV & Xpert Xpress Strep A

20-30 minutes to result Flu A/B, RSV

18-24 minutes to result Strep A

RT-PCR

Interpreted by instrument

Flu/RSV: CLIA-waived for use with nasal/nasopharyngeal swabs

For in vitro Diagnostic Use

cobas® LIAT® - Lab In a Tube (Roche)

- 20 minutes to results Flu A/B, RSV
- 15 minutes to results Strep A
- RT-PCR
- Interpreted by instrument

Flu: CLIA-waived for use with nasopharyngeal swabs

For in vitro Diagnostic Use
Silaris™ Influenza A&B Test (Sekisui)

30 minutes or less for flu A & B

RT-PCR amplification followed by hybridization and colorimetric visualization of amplified products on a test strip flu A & B

Results are interpreted visually by the operator

Flu: CLIA-waived for use with nasal swabs

For in vitro Diagnostic Use

https://www.sekisuidiagnostics.com/products-all/silaris-influenza-ab-test/
Molecular testing pros and cons

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can amplify genome</td>
<td>Typically costs more</td>
</tr>
<tr>
<td>Highly sensitive and specific</td>
<td>Takes longer</td>
</tr>
</tbody>
</table>
## Comparison of Methods

<table>
<thead>
<tr>
<th></th>
<th>POCT Rapid Antigen</th>
<th>Culture</th>
<th>Laboratory Molecular</th>
<th>POCT Molecular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Convenient</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Actionable Results</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>POCT- Friendly</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Little/No Subjectivity</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>LIS/EMR Interfaced</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>High Sensitivity/Specificity</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Low Cost</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Specific Benefits of POC Molecular Testing in a resource-limited setting:

• Drastically improved TAT- no need to send to a reference lab for confirmation (e.g. GAS culture NOT needed)
  • Getting results in real-time to act upon them

• Same testing SOC that would be found at tertiary center
  • clinical confidence in test method/results for diagnosis

• Clinic can perform testing that could previously only be done by specialized testing staff

• Test accuracy and healthcare efficiency/lab stewardship

• Offering new technology for community and outreach
Another big change has happened lately....

Clinical Practice Guidelines by the Infectious Diseases Society of America: 2018 Update on Diagnosis, Treatment, Chemoprophylaxis, and Institutional Outbreak Management of Seasonal Influenza

Last update was 2009-
BEFORE the 2009 N1H1 pandemic
and BEFORE any CLIA-waived
molecular options at point-of-care.
What Test(s) Should Be Used to Diagnose Influenza?

**Recommendations**

10. Clinicians should use rapid molecular assays (i.e., nucleic acid amplification tests) over rapid influenza diagnostic tests (RIDTs) in outpatients to improve detection of influenza virus infection (A-II) (see Table 6).

11. Clinicians should use reverse-transcription polymerase chain reaction (RT-PCR) or other molecular assays over other influenza tests in hospitalized patients to improve detection of influenza virus infection (A-II) (see Table 6).

15. Clinicians should not use RIDTs in hospitalized patients except when more sensitive molecular assays are not available (A-II), and follow-up testing with RT-PCR or other molecular assays should be performed to confirm negative RIDT results (A-II).
### Table 6. Influenza Diagnostic Tests for Respiratory Specimens

<table>
<thead>
<tr>
<th>Testing Category</th>
<th>Method</th>
<th>Influenza Viruses Detected</th>
<th>Distinguishes Influenza A Virus Subtypes</th>
<th>Time to Results</th>
<th>Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid molecular assay</td>
<td>Nuclie acid amplification</td>
<td>Influenza A or B viral RNA</td>
<td>No</td>
<td>15-30 minutes</td>
<td>High sensitivity; high specificity</td>
</tr>
<tr>
<td>Rapid influenza diagnostic test</td>
<td>Antigen detection</td>
<td>Influenza A or B virus antigens</td>
<td>No</td>
<td>10-15 minutes</td>
<td>Low to moderate sensitivity (higher with analyzer device); high specificity;</td>
</tr>
<tr>
<td>Direct and indirect immunofluorescence assays</td>
<td>Antigen detection</td>
<td>Influenza A or B virus antigens</td>
<td>No</td>
<td>1-4 hours</td>
<td>Moderate sensitivity; high specificity</td>
</tr>
<tr>
<td>Molecular assays (including RT-PCR)</td>
<td>Nuclie acid amplification</td>
<td>Influenza A or B viral RNA</td>
<td>Yes, if subtype primers are used</td>
<td>1-9 hours</td>
<td>High sensitivity; high specificity</td>
</tr>
<tr>
<td>Multiplex molecular assays</td>
<td>Nuclie acid amplification</td>
<td>Influenza A or B viral RNA, other viral or bacterial targets (RNA or DNA)</td>
<td>Yes, if subtype primers are used</td>
<td>1-2 hours</td>
<td>High sensitivity; high specificity</td>
</tr>
<tr>
<td>Rapid cell culture (shell vial and cell mixtures)</td>
<td>Virus isolation</td>
<td>Influenza A or B virus</td>
<td>Yes</td>
<td>1-3 days</td>
<td>High sensitivity; high specificity</td>
</tr>
<tr>
<td>Viral culture (tissue cell culture)</td>
<td>Virus isolation</td>
<td>Influenza A or B virus</td>
<td>Yes</td>
<td>3-10 days</td>
<td>High sensitivity; high specificity</td>
</tr>
</tbody>
</table>

Negative results may not rule out influenza. Respiratory tract specimens should be collected as close to illness onset as possible for testing. Clinicians should consult the manufacturer's package insert for the specific test for the approved respiratory specimens. Most US Food and Drug Administration (FDA)-cleared influenza diagnostic tests are approved for upper respiratory tract specimens but not for sputum or lower respiratory tract specimens. Specificities are generally high (>90%) for all tests compared to RT-PCR. FDA-cleared rapid influenza diagnostic tests are Clinical Laboratory Improvement Amendments (CLIA)-waived; most FDA-cleared rapid influenza molecular assays are CLIA-waived, depending on the specimen.

Abbreviation: RT-PCR, reverse transcription polymerase chain reaction.
During low flu activity positive RIDTs should be confirmed by molecular assay. During high flu activity negative RIDTs should be confirmed by molecular assay.
GAS study goals

Compare the BD Veritor™, Alere i™, and culture for detection of GAS

Evaluate the hypothetical impact of results on antibiotic utilization

Berry et al., J. Clin. Microbiol., 2018
Study design

• Prospectively tested 216 clinical throat samples that were collected during the months of May and June of 2016 for routine strep throat testing from two predominantly pediatric outpatient clinics within our hospital system.

• Routine patient testing (BD Veritor™ with reflex to group A strep culture) was performed and compared to results obtained on the ID NOW™ (formerly Alere™ i) system.

• Inclusion criteria was a strep throat test ordered by a clinician. Pediatric cases (<18 years of age) accounted for 199 (92.1%) of the specimens, while adults (≥18 years of age) accounted for 17 (7.9%) of the specimens.

• Each patient was subjected to two Rayon throat (posterior oropharynx) swabs as a part of their routine strep throat workup in the clinic. BD Veritor™ testing was performed in the clinic where patients were initially seen.
Study Design

Clinic

Swab 1 -> Result

Lab

Swab 2 -> aliquot

RT-PCR (for discordants) -> Result

Result

Berry et al., J. Clin. Microbiol., 2018
Distribution of positive results

Distribution of positive results

*Assay adjudication was done for each of the single-assay positive results 0/5 (0%) of BD Veritor™ and 8/9 (89%) of the ID NOW™, were confirmed by RT-PCR

Berry et al., J. Clin. Microbiol., 2018
Table 1: Sensitivity, Specificity, Accuracy, and Kappa Index analysis of each assay

<table>
<thead>
<tr>
<th>Assay</th>
<th>POSITIVE</th>
<th>NEGATIVE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alere i™</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>42</td>
<td>15</td>
<td>57</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>158</td>
<td>158</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>173</td>
<td>215</td>
</tr>
<tr>
<td>Sensitivity (95% CI) (%)</td>
<td>100.0 (91.6, 100.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity (95% CI) (%)</td>
<td>91.3 (86.1, 95.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accuracy (95% CI) (%)</td>
<td>93.0 (88.8, 96.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kappa Index</td>
<td>0.805 (0.711, 0.898)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kappa Index P-value</td>
<td>&lt;.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Veritor™</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>32</td>
<td>11</td>
<td>43</td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
<td>162</td>
<td>172</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>173</td>
<td>215</td>
</tr>
<tr>
<td>Sensitivity (95% CI) (%)</td>
<td>76.2 (60.5, 87.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity (95% CI) (%)</td>
<td>93.6 (88.9, 96.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accuracy (95% CI) (%)</td>
<td>90.2 (85.5, 93.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kappa Index</td>
<td>0.692 (0.569, 0.815)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kappa Index P-value</td>
<td>&lt;.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Table 2: Sensitivity, Specificity and Accuracy of RT-PCR Adjudicated Results

<table>
<thead>
<tr>
<th>Assay</th>
<th>Culture + RT-PCR Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POSITIVE</td>
</tr>
<tr>
<td><strong>Alere i™</strong></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>56</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100.0 (%)</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.4 (%)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>99.5 (%)</td>
</tr>
<tr>
<td><strong>Veritor™</strong></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>37</td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>78.7 (%)</td>
</tr>
<tr>
<td>Specificity</td>
<td>96.4 (%)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>92.6 (%)</td>
</tr>
</tbody>
</table>

Alere i™: 14/15 confirmed by RT-PCR  
Veritor™: 5/11 confirmed by RT-PCR
Antibiotics chart review

73/215 (34%) patients given antibiotics at the time of clinic visit

26/73 (36%) treatment inappropriate - confirmed GAS negative result

• In 20/26 (77%) cases, ALL tests were negative

• All 5 false positive BD Veritor™ results were treated with antibiotics

• 19% (5/26) of inappropriately treated cases

13/215 (6%) cases where the BD Veritor™ result was negative and antibiotics were not started at the time of the clinic visit, but that were subsequently detected by RT-PCR

• Alere i™ result was positive in 13/13 (100%) of these same cases

• In 6/13 (46%) cases, the antibiotics were started 2-6 days after the clinic visit, after receiving culture results

Berry et al., J. Clin. Microbiol., 2018
Summary - GAS study

• The Alere i™ had higher sensitivity and specificity when compared to BD Veritor™

• RT-PCR showed that none of the 5 positives (0%) detected only by the BD Veritor™ confirmed, while 8/9 (89%) of positives detected by the Alere i™ confirmed

• 36% (n=26) of patients who were given abx had no GAS identified. Of this group 19% (n=5) had false-positive BD Veritor™ results

Berry et. al, J. Clin. Microbiol., 2018
Summary – GAS study (continued)

• 6% (n=13) of positive cases were missed by the BD Veritor™, while the Alere i™ detected all 13 (100%) cases.

• Antibiotics were started 2-6 days after the visit in 6 (46%) cases, with one patient lost to documented follow-up.

• The remaining 6 (46%) patients were culture negative and were therefore not treated, but were RT-PCR confirmed as positive. Use of the Alere i™ assay could have potentially led to these 6 (100%) missed patients being treated.

Berry et. al, J. Clin. Microbiol., 2018
Conclusions of GAS study

• The Alere i™ had superior performance over the BD Veritor™

• More accurate results could assist in better utilization of antibiotics in real time

• Molecular platforms should be considered as viable alternative POCT devices for diagnosis of GAS pharyngitis

Berry et. al, J. Clin. Microbiol., 2018
Overall conclusions

• There are now user-friendly, CLIA-waived molecular testing platforms available for POCT.

• These new platforms are designed to accommodate almost any skill set and testing environment

• Molecular testing methodologies have the ability to drastically improve diagnostic turnaround times, increase overall testing accuracy, and drive more appropriate therapy choices for better patient outcomes
Thank you!

Questions?

Gregory J. Berry, Ph.D., D(ABMM)
Assistant Professor, Pathology and Laboratory Medicine
Zucker School of Medicine at Hofstra/Northwell

Director of Molecular Diagnostics and Microbiology
Northwell Health Labs and Long Island Jewish Medical Center

Assistant Director, Infectious Disease Diagnostics