The Application of molecular POCT for Influenza and Group A Strep Detection

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Objectives

- Introduce Point-of-Care Testing (POCT) uses in diagnosis of infectious diseases
- Explain the difference between molecular POCT and traditional antigen-based assays
- Review different POCT methodologies and instruments for Influenza and group A strep
- Present data from molecular Influenza and group A strep studies done in the POCT arena
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
Historical impediments to POCT

- 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 to POCT barriers

<table>
<thead>
<tr>
<th>Problems</th>
<th>Solutions</th>
</tr>
</thead>
</table>
| - Not accurate enough for definitive diagnosis  
  * E.g. rapid strep and flu tests | - Increasing sensitivity and specificity  
  * Molecular testing |
| - Too difficult to perform at point-of-care  
  * E.g. molecular testing | - Assays designed to be user-friendly and more error-proof |
| - Too Expensive | - Costs decreasing over time and reimbursement that matches test costs |
POCT in infectious disease diagnostics

• These are 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

Antiviral Drugs Most Effective during first 2-4 days

Rapid POCT Sensitivity Highest

Days Post Symptom Onset

0 1 2 3 4 5 6 7 8 9 10

High Viral Titer

Low Viral Titer
So is proper specimen collection!

C. Satzke et al. / Vaccine 32 (2014) 165–179
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 (NEW)
Rapid antigen detection tests

- Immunoassays—viral/bacterial antigens
- Qualitative resulting
- Vary greatly in their sensitivity
  - Negative strep a results need culture confirmation
  - RIDTs reclassified to class II
What changed with rapid influenza virus antigen detection tests (RIDTs)?

• **These tests were classified as Class I devices**
  - General controls were considered sufficient

• **FDA has re-classified them to Class II**
  - Both general and special controls must now be followed
Microbiology Devices; Reclassification of Influenza Virus Antigen Detection Test Systems Intended for Use Directly With Clinical Specimens

A Rule by the Food and Drug Administration on 01/12/2017

AGENCY:
Food and Drug Administration, HHS.

ACTION:
Final order.

SUMMARY:
The Food and Drug Administration (FDA) is reclassifying antigen based rapid influenza virus antigen detection test systems intended to detect influenza virus directly from clinical specimens that are currently regulated as influenza virus serological reagents from class I into class II with special controls and into a new device classification regulation.
Why the change with flu RIDTs?

- During the H1N1 influenza pandemic of 2009, questions were raised about the sensitivity of RIDTs
  - Lower sensitivity than package insert

- Concerns raised about the overall quality of influenza testing

- **Overall goal:** lower the number of misdiagnosed influenza infections by increasing the number of devices that can reliably detect the influenza virus

https://www.federalregister.gov/d/2017-00199/p-19
Minimum acceptance criteria

Sensitivity

**Flu A**  Point estimate of 90% with 80% lower bound of the 95% confidence interval  
**Flu B**  Point estimate of 80% with 70% lower bound of the 95% confidence interval  

Specificity

All influenza detection devices should demonstrate specificity with a lower bound of the 95% confidence interval exceeding 90% for both, Flu A and Flu B.

b. **When compared to a molecular comparator method:**

Sensitivity

**Flu A**  Point estimate of 80% with 70% lower bound of the 95% confidence interval  
**Flu B**  Point estimate of 80% with 70% lower bound of the 95% confidence interval  

Specificity

All influenza detection devices should demonstrate a specificity estimate with a lower bound of the 95% confidence interval exceeding 90% for both, influenza A and influenza B.
Molecular POCT
Molecular POCT tests for infectious diseases

• Traditionally designated by CLIA as moderate/high complexity and have been performed in the clinical laboratories
  • Only rapid antigen testing was available as CLIA waived

• CLIA waived tests have recently become available
CLIA waived molecular tests for infectious diseases

- **January 8th, 2015**: First CLIA waived test for influenza A and B (Alere i Influenza A&B)

- Followed by the Roche cobas Influenza A/B

- Both of these tests are classified as class II, so they are already compliant

Group A Strep and RSV are also now available on both platforms
## 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>
## Technology comparison

<table>
<thead>
<tr>
<th></th>
<th>IMMUNOASSAY</th>
<th>MOLECULAR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RAPIDS</td>
<td>LAT FLOW READERS</td>
</tr>
<tr>
<td>FAST</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>CONVENIENT</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>POC-FRIENDLY</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>ACTIONABLE RESULTS</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>REMOVES SUBJECTIVITY</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>CONNECTED</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>EXCELLENT PERFORMANCE</td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>
The power of sample amplification

Detection threshold

Amplified Flu+ Sample

Not Amplified Flu+ Sample
Molecular tests on the market

PCR – Polymerase Chain Reaction

• Rely on the ability to amplify due to temperature cycling
• Many traditional molecular companies
• Alere q - Competitive Reporter Amplification
• Cepheid – GeneExpert
• Roche LIAT – Lab in a tube

Isothermal

• Rely on the ability to do the reaction at a single temperature
• Meridian’s LAMP (loop mediated isothermal amplification)
• Quidel Solana – HDA (Helicase dependent amplification)
• Alere i – NEAR / RPA (Nicking enzyme amplification rxn/Recombinase polymerase amplification)
Alere™ i

8-13 minutes to result for Flu/RSV

4-8 minutes to result for Strep A

< 2 minutes hands on time

Small footprint (8.15” W x 5.71” H x 7.64” D)

Weight= 1.4 lbs / 3 kg

FDA-cleared for use with both nasal swabs (direct) and NP or nasal swabs in VTM
CLIA-waived for use with nasal swabs (direct) only
LIAT - Lab In a Tube

- 20 minutes to results Flu/RSV
- 15 minutes to results Strep A
- Footprint 4.5 x 9.5 x 7.5
- Weight 8.3 lbs

CLIA-waived by FDA for use with nasopharyngeal swabs only
INFLUENZA A/B STUDY
Comparison of the Alere i and BD Veritor Assays for the Rapid Detection of Influenza A and B Viruses

Gregory J. Berry, Olajumoke Oladipo, Debbie Wittnebert, Michael J. Loeffelholz, and John R. Petersen

Background: The use of point-of-care testing (POCT) in patient management decisions is becoming increasingly common. Our goal was to evaluate the diagnostic performance of 2 commercially available rapid POCT devices for influenza viruses A and B: the Alere™ i Instrument (Alere, Scarborough) and the BD Veritor™ System (BD Diagnostics).

Methods: Paired nasopharyngeal swabs were collected from patients (18–71 years) presenting with influenza-like symptoms at 3 outpatient clinics. A total of 65 samples were obtained. The Alere i and BD Veritor were performed according to the manufacturers' instructions. Discordant results were resolved using real-time reverse transcription PCR (RT-PCR).

Results: In a head-to-head comparison involving symptomatic adult patients visiting outpatient clinics during the 2014–2015 and 2015–2016 influenza seasons, the Alere i and BD Veritor had 90.63% agreement in the detection of influenza A virus and a statistically significant observed κ coefficient of 0.754 (P <0.0001). Discordant results between the Alere i and BD Veritor were further investigated using RT-PCR, showing that the BD Veritor missed 5 positive influenza A virus results (false negatives) and detected 1 false positive, while the Alere i results agreed with all RT-PCR results. There were no discordant results between the Alere i and BD Veritor in the detection of influenza B virus.

Conclusions: Our data suggest that the Alere i has higher sensitivity and specificity than the BD Veritor in the detection of influenza A virus. Both assays showed equal performance in the detection of influenza B virus.
Goal

- Evaluate the diagnostic performance of 2 commercially available rapid POCT devices for influenza viruses A and B:
Study design

• Paired nasopharyngeal swabs were collected from patients (18–71 years) presenting with influenza-like symptoms at 3 outpatient clinics
  • A total of 65 samples were obtained

• The Alere i and BD Veritor were performed according to the manufacturers' instructions

• Discordant results were resolved using real-time reverse transcription PCR (RT-PCR)
Table 1. Comparison of the Alere I and BD Veritor in the detection of Influenza A and B viruses.

<table>
<thead>
<tr>
<th></th>
<th>Alere i</th>
<th>BD Veritor</th>
<th>BD Veritor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influenza A</td>
<td>Influenza B</td>
<td>Influenza B</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>Agreement %</td>
<td>90.63</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Observed k, linear weighting</td>
<td>0.754, 95% CI 0.569-0.938</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.0001</td>
<td></td>
<td>0.00</td>
</tr>
</tbody>
</table>
Results

• Influenza A:
  • RT-PCR was done on discordants
    • BD Veritor missed 5 positive results (false negatives); detected 1 false positive result
    • Alere i agreed with all RT-PCR results
  
• Influenza B:
  • No discordant results

One Alere i invalid was also excluded from analysis, but was positive by the BD Veritor and confirmed by RT-PCR.
Conclusions

• The Alere i has higher sensitivity and specificity than the BD Veritor in the detection of influenza A virus

• Both assays showed equal performance in the detection of influenza B virus
GROUP A
STREPTOCOCCUS STUDY
Group A Strep study goal:

• Compare the BD Veritor, Alere i, and culture for detection of Group A Streptococcus
• Evaluate the hypothetical impact of results on antibiotic utilization

RIDT with reader  
BD Veritor™  
Alere™ i  
Isothermal amplification  
Culture
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 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.
Distribution of positive results

Culture

0

Alere i

10

6

9

BD Veritor

0

32

5

Berry et. al, J. Clin. Microbiol. JCM.01310-17; Accepted manuscript posted online 5 January 2018
Table 2: Agreement between the Alere i and BD Veritor

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Pos</th>
<th>Neg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alere</td>
<td>Pos</td>
<td>38</td>
<td>19</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Neg</td>
<td>5</td>
<td>153</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>43</td>
<td>172</td>
<td>215</td>
</tr>
<tr>
<td>Agreement</td>
<td>.</td>
<td>.</td>
<td></td>
<td>0.888</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(95% CI 0.838-0.927)</td>
</tr>
<tr>
<td>Kappa Index</td>
<td>.</td>
<td>.</td>
<td></td>
<td>0.689</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(95% CI 0.575-0.803)</td>
</tr>
<tr>
<td>P-value</td>
<td>.</td>
<td>.</td>
<td></td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Berry et. al, *J. Clin. Microbiol. JCM.01310-17; Accepted manuscript posted online 5 January 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>Alere i</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>
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<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>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Assay</th>
<th>POSITIVE</th>
<th>NEGATIVE</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Veritor</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>
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<tr>
<td>Accuracy (95% CI) (%)</td>
<td>90.2 (85.5, 93.9)</td>
<td></td>
<td></td>
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<tr>
<td>Kappa Index</td>
<td>0.692 (0.569, 0.815)</td>
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<td></td>
</tr>
<tr>
<td>Kappa Index P-value</td>
<td>&lt;.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Assay</th>
<th>POSITIVE</th>
<th>NEGATIVE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alere i Positive</td>
<td>56</td>
<td>1</td>
<td>57</td>
</tr>
<tr>
<td>Alere i Negative</td>
<td>0</td>
<td>158</td>
<td>158</td>
</tr>
<tr>
<td>Alere i Total</td>
<td>56</td>
<td>159</td>
<td>215</td>
</tr>
<tr>
<td>Sensitivity (95% CI) (%)</td>
<td></td>
<td></td>
<td>100.0 (93.6, 100.0)</td>
</tr>
<tr>
<td>Specificity (95% CI) (%)</td>
<td></td>
<td></td>
<td>99.4 (96.6, 99.9)</td>
</tr>
<tr>
<td>Accuracy (95% CI) (%)</td>
<td></td>
<td></td>
<td>99.5 (97.4, 99.9)</td>
</tr>
<tr>
<td>Veritor Positive</td>
<td>37</td>
<td>6</td>
<td>43</td>
</tr>
<tr>
<td>Veritor Negative</td>
<td>10</td>
<td>162</td>
<td>172</td>
</tr>
<tr>
<td>Veritor Total</td>
<td>47</td>
<td>168</td>
<td>215</td>
</tr>
<tr>
<td>Sensitivity (95% CI) (%)</td>
<td></td>
<td></td>
<td>78.7 (64.3, 89.3)</td>
</tr>
<tr>
<td>Specificity (95% CI) (%)</td>
<td></td>
<td></td>
<td>96.4 (92.4, 98.7)</td>
</tr>
<tr>
<td>Accuracy (95% CI) (%)</td>
<td></td>
<td></td>
<td>92.6 (88.2, 95.7)</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

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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
Summary- 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 and the cobas Liat would have led to 4/6 (67%) of these patients being treated.

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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
Overall conclusions

• Infectious disease testing will continue to enter the POCT

• Molecular POCT is as sensitive/specific as most lab tests, but has the huge advantage of a rapid answer

• These tests have the ability to drive more appropriate therapy choices for better patient outcomes
Acknowledgements

• UTMB Dept. of Pathology
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  • Dr. Christopher Marquez
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  • Ms. Peggy Mann

• Memorial Medical Center Hospital
  • Ms. Debbie Wittnebert
THANK YOU!

Questions?