Molecular Diagnostics & Point of Care Testing

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Adjunct Associate Professor of Pathology, Mount Sinai School of Medicine

Meeting Name
Location

day | month | year
Disclaimers

- I am a salaried employee of Abbott Rapid Diagnostics
- ARDx produces molecular testing devices for use at the POC
- All data in this presentation can be found in product labeling or peer reviewed literature
- This will be a balanced presentation describing the characteristics of various molecular POCT systems
Advantages of Rapid Testing for Infectious Diseases

Faster directed therapy to reduce:
- antibiotic resistance
- hospital length-of-stay

Less adverse consequences

Teachable moment

Reduced length-of-stay in Emergency Department

Timely application of appropriate infection control procedures
What are the issues of respiratory disease?

**The symptoms of respiratory diseases are vague**
- Pneumonia symptoms  
  - Cough  
  - Fever  
  - Chills  
  - Difficulty breathing
- Influenza  
  - Cough  
  - Fever  
  - Chills  
  - Malaise

**Treatment is different**
- Bacteria  
  - Broad spectrum antibiotic  
  - Narrow spectrum antibiotic
- Influenza  
  - Antiviral  
  - Treat symptoms only

**Complications of mistreatment**
- Mistreatment of bacterial etiology  
  - May increase morbidity/mortality  
  - May have longer hospital stay  
  - May get *C. difficile*
- Mistreatment of influenza  
  - May have increased resistance and *C. difficile*
Results – Flu Negative

<table>
<thead>
<tr>
<th>Procedure</th>
<th>MD unaware, n=92</th>
<th>MD aware, n=97</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBC</td>
<td>7 13</td>
<td>7 6</td>
</tr>
<tr>
<td>Blood Culture</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Urine Dipstick</td>
<td>7 7</td>
<td>8 10</td>
</tr>
<tr>
<td>Urinalysis</td>
<td>8 10</td>
<td>5 12</td>
</tr>
<tr>
<td>Urine culture</td>
<td>5 12</td>
<td>2 3</td>
</tr>
<tr>
<td>CSF studies/culture</td>
<td>2 3</td>
<td>23 22</td>
</tr>
<tr>
<td>Chest X-ray</td>
<td>27 27</td>
<td>27 27</td>
</tr>
<tr>
<td>Antibiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antivirals</td>
<td>2 0</td>
<td></td>
</tr>
</tbody>
</table>


FOR EXTERNAL USE, PRINT/DISTRIBUTION PERMITTED
Results – Flu Positive


FOR EXTERNAL USE, PRINT/DISTRIBUTION PERMITTED
## Key Operational Metrics

<table>
<thead>
<tr>
<th>FLU POSITIVE</th>
<th>FLU NEGATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lab/Rad Charges</strong>*</td>
<td><strong>$92.37</strong></td>
</tr>
<tr>
<td>MD unaware, n = 106</td>
<td><strong>$15.65</strong></td>
</tr>
<tr>
<td>MD aware, n = 96</td>
<td><strong>49</strong></td>
</tr>
<tr>
<td>Time to Discharge (min)*</td>
<td><strong>25</strong></td>
</tr>
</tbody>
</table>


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Is the pathogen bacterial or viral? Influenza and pneumonia symptoms can overlap dramatically.

Who do you test? If it is flu season, do you test for other pathogens?

What do you test them for? Different age groups are linked to different pathogens.

Can treatment be impacted if the appropriate testing is done? Stop indiscriminate use of broad spectrum antibiotics.
Misuse of Antibiotics Can Lead to Other Medical Issues

<table>
<thead>
<tr>
<th>Respiratory issues can be treated with fluoroquinolone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disrupts normal intestinal flora</td>
</tr>
<tr>
<td><strong>O27 strain of <em>C. difficile</em> is specifically resistant to fluoroquinolone</strong></td>
</tr>
</tbody>
</table>
Issues with Clinical Samples

- Viral titer is highest in first 48 hours
- Proper sample collection is necessary
- Dilution in transport media
Rapid Tests

Pro

- Tests take minimal time
- Some tests are so simple that they are CLIA-waived
- Can be used to triage patients
- Positive results can be used to rule out other issues like pneumonia so don’t give unnecessary chest x-ray, antibiotics, etc.

Con

- Performance is not as good as culture, PCR, or DFA
- Often used as a screening test, usually with negatives requiring additional confirmation.
Molecular Tests

Pro

• For respiratory specimens, high performance
• Same day results

Con

• Turn around time from lab may be extensive, especially if batching specimens
• Expensive
• May require experienced technicians, labs, dedicated equipment, etc.
## Pros and Cons of Molecular Testing

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good for pathogens that you only have when you are sick</td>
<td></td>
</tr>
<tr>
<td>• Influenza</td>
<td></td>
</tr>
<tr>
<td>Good for living things which would have RNA/DNA</td>
<td></td>
</tr>
<tr>
<td>Bad for non living things</td>
<td></td>
</tr>
<tr>
<td>• Protein, DOA</td>
<td></td>
</tr>
<tr>
<td>Good to see if active infection &amp; can test where the infection is</td>
<td></td>
</tr>
<tr>
<td>• Not things like sepsis</td>
<td></td>
</tr>
<tr>
<td>May only be a screen for bacteria/viruses that people may normally carry</td>
<td></td>
</tr>
<tr>
<td>• <em>Clostridium difficile</em></td>
<td></td>
</tr>
<tr>
<td>Bad for past infection</td>
<td></td>
</tr>
<tr>
<td>• Want test that detects antibody</td>
<td></td>
</tr>
</tbody>
</table>
Molecular Tests on the Market

PCR – Polymerase Chain Reaction

- Rely on the ability to amplify due to temperature cycling
- Many traditional molecular companies, e.g,
  - **Alere™ q** - Competitive Reporter Amplification
  - Cepheid GeneXpert®
  - BD Affirm™ VPIII – direct probe
  - Biocartis Idylla™ – qPC
  - Enigma® MiniLab™ – qPCR - Flu A/B, RSV
  - Roche cobas® Liat – Lab in a tube
  - Spartan RX (PGx) – PCR

Isothermal

- Rely on the ability to do the reaction at a single temperature
- Meridian **illumi**gene® - LAMP (loop mediated isothermal amplification)
- Quidel Solana® – HDA (Helicase dependent amplification)
- **Alere™ i** – NEAR (Nicking enzyme amplification reaction)
# Introducing the Players in PCR

<table>
<thead>
<tr>
<th>Patient sample containing DNA (or RNA)</th>
<th>Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>• May or may not have target gene</td>
<td>• short bits of manufactured DNA that recognize the target gene</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nucleotides</th>
<th>Taq Polymerase</th>
</tr>
</thead>
<tbody>
<tr>
<td>• building blocks of DNA</td>
<td>• Enzyme that replicates DNA in a PCR reaction</td>
</tr>
</tbody>
</table>

**Fluorescent dye for reporting results**
- realtime PCR
PCR Cycle

Double-stranded DNA

95° Denaturation

Heating separates strands

57° Annealing

Taq Polymerase Binds at Primer Sites

Taq Polymerase reads existing DNA strand to create a new matching one

72° Extension
PCR Amplification

DNA doubles every cycle

1 Cycle

2 Cycles

3 Cycles

4 Cycles

After 30 cycles = 1,073,741,824 copies

After 40 cycles = 1,099,511,627,776 copies
Roche cobas® Liat - Lab In a Tube

20 minutes to results Flu

15 minutes to results Strep A

Footprint 4.5 x 9.5 x 7.5

Weight 8.3 lbs

Flu A/B
- Sensitivity 100%/100%
- Specificity 96.8%/94.1%
- LOD $10^{-2} - 10^{-1} / 10^{-3} - 10^{-1}$ TCID$_{50}$/mL

Strep A
- Sensitivity 98.3%
- Specificity 94.2%
- LOD 5-20 CFU/mL

RSV
- Sensitivity 97.0%
- Specificity 98.7%
- LOD 4 CFU/mL

Data sourced from Roche Product Labeling
Permission granted by Roche Diagnostics
Sample processing in the Liat Tube

Liat HIV Quant Assay amplification plot


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Linearity of the Liat HIV Quant Assay

The Alere™ q

Portable bench-top real time (rt) Reverse Transcriptase (RT) PCR system for processing and analysis of Alere q HIV-1/2 test cartridges

50 minutes to results

7.8 kg (3.5 lbs)

In-built battery to seamlessly bridge power outages

Not Available in US
The Alere™ q HIV-1/2 Detect Cartridge

- Qualitative measurements of HIV-1 (subtypes M/N and O) and HIV-2
- Low sample volume - only 25 µl of capillary/EDTA venous whole blood or plasma
- All reagents and controls enclosed in the test cartridge
- No manual sample processing
- Fully automated capture and enrichment of the specific RNA target, reverse transcription and real time PCR
- High speed target amplification and real time multiplex detection based on CMA (Competitive reporter Monitored Amplification) assay format
Competitive Reporter Monitored Amplification

A

B

C

Test Results:

For HIV-1 (subtypes M/N and O) and HIV-2 a **qualitative** (detected/undetected) result is given.

QC Parameters:

Sample Detection: control for sufficient sample volume
Device: multiple QC parameters for the functionality of Alere™ q
HIV-1 Positive Control: internal amplification control for HIV-1
HIV-2 Positive Control: internal amplification control for HIV-2
Negative Control: control for non-specific hybridization
Analysis: multiple QC parameters for the Analysis process, incl. positive hybridization control

Failing of at least one of these controls renders the test invalid.
Alere™ q HIV 1/2: Mozambique EID Study

- Blinded cross-sectional study of 827 HIV-exposed infants (1-18 months)
- **Alere™ q** HIV 1/2 performed by nurses at POC in 4 primary health care centres and 1 hospital ward
- Reference Method: Roche Diagnostics PCR at the reference laboratory

<table>
<thead>
<tr>
<th>AGE</th>
<th>Overall</th>
<th>HIV-pos*</th>
<th>%-pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2m</td>
<td>500</td>
<td>19</td>
<td>3.8%</td>
</tr>
<tr>
<td>2-3m</td>
<td>124</td>
<td>6</td>
<td>4.8%</td>
</tr>
<tr>
<td>3-6m</td>
<td>111</td>
<td>12</td>
<td>10.8%</td>
</tr>
<tr>
<td>6-9m</td>
<td>58</td>
<td>14</td>
<td>24.1%</td>
</tr>
<tr>
<td>&gt;9m</td>
<td>34</td>
<td>14</td>
<td>41.1%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>827</td>
<td>65</td>
<td>7.9%</td>
</tr>
</tbody>
</table>

* HIV-positivity defined by the Roche technology

Data sourced from Jani et al., J Acquir Immune Defic Syndr Volume 67, Number 1, September 1, 2014

<table>
<thead>
<tr>
<th>Positives</th>
<th>Negatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>POC NAT Results</td>
<td>64</td>
</tr>
<tr>
<td>Conventional Results</td>
<td>1</td>
</tr>
</tbody>
</table>
GeneXpert® - Cepheid

- 75 minutes to results
  - 2 min hands on time
- Broad molecular menu
  - 11 FDA approved assays*
- Footprint 3 x 4.2 x 9.1"
- 2.2 lbs
- Battery powered

Not Yet Available

Data sourced from Cepheid Product Labeling
Permission granted by Cepheid

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Isothermal Molecular Technologies

cHDA : Circular Helicase-dependent amplification
HDA : Helicase-dependent amplification
IMDA : Isothermal multiple displacement amplification
LAMP : Loop-mediated isothermal amplification
MPRCA : Multiply-primed rolling circle amplification
NASBA : Nucleic acid sequence based amplification
NEAR: Nicking enzyme amplification reaction
RAM : Ramification amplification method
RCA : Rolling circle amplification
RPA : Recombinase polymerase amplification
SDA : Strand displacement amplification
SMART : Signal mediated amplification of RNA technology
SPIA : Single primer isothermal amplification
TMA : Transcription mediated amplification
Isothermal Molecular Technologies

**cHDA** : Circular Helicase-dependent amplification  
**HDA**  : Helicase-dependent amplification  
**IMDA**  : Isothermal multiple displacement amplification  
**LAMP**  : Loop-mediated isothermal amplification  
**MPRCA**  : Multiply-primed rolling circle amplification  
**NASBA**  : Nucleic acid sequence based amplification  
**NEAR**  : Nicking enzyme amplification reaction  
**RAM**   : Ramification amplification method  
**RCA**   : Rolling circle amplification  
**RPA**   : Recombinase polymerase amplification  
**SDA**  : Strand displacement amplification  
**SMART**  : Signal mediated amplification of RNA technology  
**SPIA**  : Single primer isothermal amplification  
**TMA**   : Transcription mediated amplification
Helicase Dependent Amplification Assays

Diagram showing the process of helicase-dependent amplification.
35 minutes to results
• Including heat pretreatment step

Small footprint (9.4” x 9.4” x 5.9”)

8.8 lbs

Battery pack available

GAS only FDA approved test
• Sensitivity – 98.2%
• Specificity – 97.2%
• LOD – $6.81 \times 10^4$ CFU/mL
Step 1
Specific primers bind to target sequences that have been separated by the helicase.

Step 2
Specific DNA probes labeled with a quencher on one end and a fluorophore on the other end bind to the single-stranded biotinylated amplicons.

Step 3
Upon annealing to the amplicons, the fluorescence probes are cleaved and the fluorescence signal increases due to physical separation of fluorophore from quencher.

Data sourced from Quidel Product Labeling
Loop Mediated Amplification

Use of 4–6 different primers to recognize 6-8 distinct regions

Outer primers are known as F3 and B3

Inner primers are forward inner primer (FIB) and backward inner primer (BIP)

Reprinted from Trends in Parasitology, 31/8, Alhassan, Li, Poole, Carlow, Expanding the MDx toolbox for filarial diagnosis and surveillance, 391-400, (2015), with permission from Elsevier
LAMP final products are stem loop DNAs

The final products are stem loop DNAs with several inverted repeats of the target and cauliflower-like structures with multiple loops due to hybridization between alternately inverted repeats in the same strand

Positive LAMP reactions can be visualized with the naked eye
illumi\textsuperscript{gene}® – Meridian Bioscience

- **< 60 minutes to results**
  - Including heat pretreatment step
- **< 2 minutes hands on time**
- **Small footprint (8.3” x 11.5” x 3.7”)**
- **6.5 lbs**
- **Room temp storage**
- **6 FDA approved tests – C. difficile, GAS, GBS, HSV 1&2, Mycoplasma, Pertusis**
  - GAS Sensitivity – 98.0%
  - GAS Specificity – 97.7%
  - GAS LOD – 400-430 CFU/mL

Data sourced from Meridian Bioscience Product Labeling
NEAR Mechanism – Amplification from RNA

- NEAR amplifies target sequence directly from single stranded RNA
  - No heat denaturation required
  - Reverse transcriptase, DNA polymerase & Nicking endonuclease
  - Converts single stranded RNA to single stranded DNA

![Diagram showing NEAR mechanism]

- Annealing of Template 1 to RNA target
- Extension by Reverse Transcriptase
- Digestion of RNA strand in DNA/RNA duplex by Reverse Transcriptase
- Annealing of Template 2 to DNA target

= Reverse Transcriptase
NEAR Mechanism – Amplification from dsDNA

- Assay amplifies target sequence directly from double-stranded genomic DNA
  - No heat denaturation required
  - Nicking Enzyme, DNA Polymerase
  - Creates single-strand copy of genome

\[
\begin{align*}
5' & \quad \text{Nicking Enzyme} \\
3' & \\
3' & \quad \text{DNA Polymerase} \\
5' & \\
\end{align*}
\]
NEAR Amplification Duplex – Bidirectional Amplification

NERS – ‘GAGTC’
Stabilization region

Nicking site

Bidirectional amplification

(1b)

(2b)

(3b)

(4b)

Bidirectional amplification

Bidirectional amplification

Bidirectional amplification

Specific Product 1

Specific Product 2
Alere™ i System

- < 15 minutes to results
- < 2 minutes hands on time
- Small footprint (8.15” W x 5.71” H x 7.64” D)
- 1.4 lbs / 3 kg
- 3 approved tests – Flu A/B, GAS, RSV
Alere™ i System

**Extraction**
Acidic / Basic conditioning or enzymatic (Ply C)

Place test base & sample receiver in Alere™ i

Add swab to sample receiver

Transfer sample extract to Test Base

**Amplification**

52°C NEAR

39°C RPA

**Detection**

Dual reaction tube

Dual channel fluorescence
## Flu Clinical Trial Results

### Alere™ i Influenza A & B Performance vs. Culture

<table>
<thead>
<tr>
<th></th>
<th>Flu A</th>
<th>Flu B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture +</td>
<td>Culture -</td>
</tr>
<tr>
<td>Alere™ i +</td>
<td>92</td>
<td>66</td>
</tr>
<tr>
<td>Alere™ i -</td>
<td>2</td>
<td>411</td>
</tr>
</tbody>
</table>

Sensitivity = 97.9% (92.6-99.4)
Specificity = 86.2% (82.8-89.0)

Sensitivity = 92.5% (84.6-96.5)
Specificity = 96.5% (94.5-97.8)

### Alere™ i Influenza A & B Performance vs. RT-PCR

<table>
<thead>
<tr>
<th></th>
<th>Flu A</th>
<th>Flu B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT-PCR +</td>
<td>RT-PCR -</td>
</tr>
<tr>
<td>Alere™ i +</td>
<td>147</td>
<td>11</td>
</tr>
<tr>
<td>Alere™ i -</td>
<td>8</td>
<td>464</td>
</tr>
</tbody>
</table>

Positive Percent Agreement = 94.8% (90.1-97.4)
Negative Percent Agreement = 97.7% (95.9-98.7)

Positive Percent Agreement = 98.4% (94.4-99.6)
Negative Percent Agreement = 99.4% (98.3-99.8)
## Summary of POCT nucleic acid amplification methods

<table>
<thead>
<tr>
<th></th>
<th>cobas® Liat</th>
<th>Alere™ q</th>
<th>GeneXpert®</th>
<th>Solana®</th>
<th>illumigene®</th>
<th>Alere™ i</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Technology</strong></td>
<td>PCR</td>
<td>PCR</td>
<td>PCR</td>
<td>HDA</td>
<td>LAMP</td>
<td>NEAR</td>
</tr>
<tr>
<td><strong>DNA Amplification</strong></td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td><strong>RNA amplification</strong></td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td><strong>“Denaturing” agent</strong></td>
<td>Heat</td>
<td>Heat</td>
<td>Heat</td>
<td>Helicase</td>
<td>Betaine</td>
<td>Restriction enzymes</td>
</tr>
<tr>
<td><strong>Pretreatment Required</strong></td>
<td>N</td>
<td>N</td>
<td>Y/N</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td><strong># of enzymes</strong></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Temp (°C)</strong></td>
<td>95/72/57</td>
<td>95/72/57</td>
<td>95/72/57</td>
<td>64</td>
<td>60-65</td>
<td>52</td>
</tr>
<tr>
<td><strong>Time to Result (min)</strong></td>
<td>&lt;20</td>
<td>55</td>
<td>75</td>
<td>35</td>
<td>&lt;60</td>
<td>&lt;15</td>
</tr>
<tr>
<td><strong>Multiple Amplifications</strong></td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>
Questions?

ellis.jacobs@alere.com