Molecular Testing in Infectious Diseases

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Objectives

• Describe the molecular methods available for diagnosis of infectious diseases
  – Platforms and Instrumentation
  – Tests availability

• Discuss the implementation of these assays according to hospital size, patient population and molecular expertise of laboratory staff

• Discuss their potential impact on hospital cost and patient outcome
Implementing Molecular Testing for Infectious Diseases Diagnosis

• Test Selection
  – Which is your patient population?
    • Pediatric versus Adult patients
    • Immunosuppressed patients
    • OBGYN services
    • Large ED or Outpatient population
  – Does your lab have experience in molecular testing?
  – Do you have any equipment?
  – Where the testing will be done (Micro lab, Core lab, Molecular Lab)

• Getting Approval from Administration
  – Convincing Laboratory and Upper Management

• Verification, Validation, and Implementation

Palavecino E. Make the Move to Molecular Diagnostics. MLO May 2010. 10-14
Examples of Molecular Tests by Complexity Level

- Sequencing
- Genotyping
- Quantitative PCR: Viral Loads
- Multiplex PCR
  - Respiratory, Blood Cultures and Stool Samples
- Two-Three Targets: Flu A and B, CT/GC
- One Target: Group B streptococci, MRSA, C difficile
Nucleic Acid Extraction

Amplification

Detection and Resulting

Close Systems
All steps in one instrument

Reduce need for molecular trained personnel and space. Allows testing on all shifts and improve turnaround time.

NOTE: Prevention of sample contamination is still very important in close systems. Sample preparation should be done in a separate room. Use of dedicated lab coat and changing gloves between sample is highly recommended.
Examples of Platforms/Instruments

Fully automated: Extraction, amplification and detection

GeneXpert
BD Max
Panther

Automated amplification and detection. Requires separate NA extraction

3M Integrated Cycler
LightCycler
eSensor
Illumigene
## Platforms and Assay Availability

Which platform/instrument would be suitable for my lab?

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Target Organisms and Platforms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GBS</strong></td>
<td><strong>Influenza</strong></td>
</tr>
<tr>
<td>BD</td>
<td>BD Max</td>
</tr>
<tr>
<td></td>
<td>Smart Cycler</td>
</tr>
<tr>
<td>Gene-Probe</td>
<td>SmartCycler</td>
</tr>
<tr>
<td>Cepheid</td>
<td>GeneXpert</td>
</tr>
<tr>
<td>Focus</td>
<td>3M Integrated cycler</td>
</tr>
<tr>
<td>Meridian</td>
<td>Illumigene</td>
</tr>
<tr>
<td>Roche</td>
<td></td>
</tr>
</tbody>
</table>
## Multiplex Real Time PCR for Infectious Diseases Syndromes

### Once sample
- Convenient for screening
- Reduce sample requirements
- Simplifies testing

### Appropriate collection of sample is the utmost importance

<table>
<thead>
<tr>
<th>Infectious disease</th>
<th>Ideal Test Menu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory infections</td>
<td>Viral and bacterial pathogens</td>
</tr>
<tr>
<td>Meningitis</td>
<td>HSV 1 and 2, Enterovirus</td>
</tr>
<tr>
<td>Sepsis</td>
<td>Gram positive, Gram Negative Bacteria and Yeasts</td>
</tr>
<tr>
<td>STD</td>
<td>Chlamydia tracomatis/Neisseria gonorrhoeae, HSV, HPV.</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>Bacterial, viral, and parasitic pathogens</td>
</tr>
<tr>
<td>Infection in transplant patients</td>
<td>CMV, BK, VZV, EBV.</td>
</tr>
</tbody>
</table>
Molecular Testing for Diagnosis of Clinical Syndromes

- Sepsis (Bacteremia)
- Hospital Acquired Infections
- Viral Respiratory Infections

Rapid Molecular Testing
- Improves Patient Outcomes
- Decreases Costs
Clinical Syndrome: Respiratory Infections

Advantages of molecular testing for viral infection
- Rapid antigen tests are not sensitive
- Viral culture methods are too slow and limited in viral menu
- Clinical presentation for respiratory infection is not specific
- Many viruses present simultaneously throughout the year...flu season is not just flu
- Molecular tests identify approximately 50% more viral pathogens than culture

Lower Respiratory infections: 250,000 death/year from Flu and 100,000 non Flu

Molecular Respiratory Panels

2014 Molecular Options

1. Luminex xTAG RVP 12+/RVP Fast 8
2. Film Array 17 viral targets and 3 bacterial targets
3. GenMark RVP 14+ viruses
4. Gene Probe -Prodesse ProFlu Plus/Plus subtypes
   - RSV/Influenza A/B/H1, H3, novel H1
   - Also, ProFlu 1-4, ProFlu hMPV, ProFlu Adenovirus. On SmartCycler
5. Nanosphere Verigene
   - RSV/Flu A/B/H1/H3
6. Focus – 3M
   - RSV/Flu A/B/ A H1N1 2009
7. Cepheid, Xpert Flu A/B (A/H1N1 2009, H1, H3)

and more…
Multiplex PCR: Detection and differentiation of respiratory viruses

- FLU A: Seasonal A/H1 or H3 2009 H1N1
- FLU B
- RSV
- Adenovirus
- Coronavirus 4 subtypes
- Rhino/Enterovirus
- hMPV
- ParaFlu 1, 2, 3 and 4

Film Array Respiratory Panel (BioFire) Detects 20 respiratory pathogens.

1. B. pertussis
2. Mycoplasma pneumoniae
3. Chlamydophila pneumoniae
Clinical Syndrome: Hospital Acquired Infections

In the U.S., HAIs affect 1.7 million patients, killing nearly 100,000 people every year.

- 273% increase in *S. aureus* HAI BSI in a study that compared 1980-83 to 1990-93
  Steinberg JP et al. CID 1996;23:255-59

- Marked increase in CDI incidence and mortality across the US specially among those ≥65 years of age.
Molecular Tests for Active Surveillance

Detection Colonized Asymptomatic Patients

Clinical cultures

MRSA

Active Surveillance
Detect colonization.

Allows:
* Treatment
* Isolation

Palavecino E. Internat ID Symposium 2007, 4:330
Detection of MRSA from Nasal Swab
Appropriate collection of nasal swab is very important

Cepheid –GeneXpert: Fully automated (sample to result). Random access

BD GeneOhm- Smart Cycler. Manual extraction, but automated amplification and detection
Results Review and Reporting

BD MRSA On Smart Cycler

Cepheid SA/MRSA on GeneXpert

Results can be transferred directly to laboratory information system.
• Inform the clinical staff about the correct interpretation of the results (S aureus versus MRSA).
• Get input from infection control. Arrange automated notification to IC.
Commercial Assays for Detection of MRSA in Nasal Swabs

<table>
<thead>
<tr>
<th>Assay</th>
<th>Company</th>
<th>Analysis Platform</th>
<th>Sens/Spec</th>
<th>Time to Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD GeneOhms MRSA ACP</td>
<td>Becton Dickinson</td>
<td>Smart Cycler System</td>
<td>92.0/94.6</td>
<td>2.5 hours</td>
</tr>
<tr>
<td>BD MAX MRSA XT</td>
<td>Becton Dickinson</td>
<td>BD MAX System</td>
<td>93.9/99.2</td>
<td>2 hours</td>
</tr>
<tr>
<td>BD MAX StaphSR*</td>
<td>Becton Dickinson</td>
<td>BD MAX System</td>
<td>93.1/97.5</td>
<td>2 hours</td>
</tr>
<tr>
<td>MRSA Advance Test</td>
<td>Roche Diagnostics</td>
<td>LightCycler</td>
<td>95.2/96.4</td>
<td>2 hours</td>
</tr>
<tr>
<td>NucliSENS EasyQ MRSA</td>
<td>BioMerieux</td>
<td>EasyQ System</td>
<td>95.8/96.8</td>
<td>3 hours</td>
</tr>
<tr>
<td>Xpert MRSA</td>
<td>Cepheid</td>
<td>GeneXpert</td>
<td>86.3/94.9</td>
<td>1 hour</td>
</tr>
<tr>
<td>Xpert SA Nasal Complete*</td>
<td>Cepheid</td>
<td>GeneXpert</td>
<td>91.9/97.9</td>
<td>1 hour</td>
</tr>
</tbody>
</table>

*: Detects *S. aureus* and MRSA

# Available FDA Cleared Assays for *Clostridium difficile*

<table>
<thead>
<tr>
<th>Assay</th>
<th>Target</th>
<th>Extraction</th>
<th>TAT</th>
<th>Cost/test</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD-GeneOhm C Diff</td>
<td><em>tcd B</em></td>
<td>Manual</td>
<td>75-90 min</td>
<td>$25-$49</td>
</tr>
<tr>
<td>Xpert C Diff</td>
<td><em>tcd B</em></td>
<td>Automated</td>
<td>45 min</td>
<td>$45</td>
</tr>
<tr>
<td>Illumigene</td>
<td><em>tcd A</em></td>
<td>Manual</td>
<td>70 min</td>
<td>$NA</td>
</tr>
<tr>
<td>Prodesse ProGastro</td>
<td><em>tcd B</em></td>
<td>Easy Mag</td>
<td>180 min</td>
<td>$25</td>
</tr>
</tbody>
</table>

Adapted from Carroll KC. Anaerobe 2011. 17: 170-174
Mortality from sepsis range from 25% to 80%
1.7 million patients annually in the US
~ $14.6 billion spent on hospitalization annually

CDC NCHS Data Brief. 2011

Early and effective therapy is crucial for patient survival of blood stream infections
Associated with a fivefold reduction in survival

Sepsis

Day 1
Traditional Methods

Day 2

Day 3-4

Empiric Treatment

Broad Spectrum

Targeted Treatment

Empiric Treatment

Targeted Treatment

Rapid tests identify the organisms 24-48 hours earlier than traditional methods

Gram stain

Multiplex PCR
Allows identification of GP and GN organisms and resistance determinants in 1-3 hours

Culture

Susceptibility

Organisms Most Commonly Isolated from Blood Cultures

- 55-60% Gram Positive Cocci
- 35-40% Gram Negative Rods

WFBMC unpublished data
Verigene (Nanosphere) BC Panel
(GP panel is FDA approved, GN panel is under evaluation)

<table>
<thead>
<tr>
<th>Organisms detected</th>
<th>Resistance markers</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td><em>mecA</em> gene</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus lugdunensis</em></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus anginosus</em></td>
<td></td>
</tr>
<tr>
<td>group</td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td><em>Van A and VanB</em></td>
</tr>
<tr>
<td><em>Enterococcus faecium</em></td>
<td></td>
</tr>
<tr>
<td><em>Micrococcus spp.</em></td>
<td></td>
</tr>
<tr>
<td><em>Listeria spp.</em></td>
<td></td>
</tr>
</tbody>
</table>

2.5 hours

Our evaluation: 98% correlation compared to culture and susceptibility testing. Six “no calls”, samples needed to be repeated.
Palavecino E et al. ICAAC 2013
**Film Array (BioFire) Blood Culture Panel (FDA approved)**

<table>
<thead>
<tr>
<th>Gram Positive</th>
<th>Gram Negative</th>
<th>Yeasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus (VRE/VSE)</td>
<td><em>P. aeruginosa</em></td>
<td>Candida glabrata</td>
</tr>
<tr>
<td>Streptococcus (Group A, B)</td>
<td>Acinetobacter</td>
<td>Candida krusei</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>Haemophilus influenza</td>
<td>Candida parasilopsis</td>
</tr>
<tr>
<td>Listeria</td>
<td>Neisseria meningitidis</td>
<td>Candida tropicalis</td>
</tr>
</tbody>
</table>

Our evaluation: 100% correlation compared to culture and susceptibility testing for GP and GN. 97% correlation for Yeasts.
Comparison of the Molecular Assays for Detection of Bacteremia

Run Time: From 1 to 3 hours

<table>
<thead>
<tr>
<th>Vendor</th>
<th>Instrument</th>
<th>Organism detected</th>
<th>Need for batching</th>
<th>Approx Cost/test</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD GeneOhm</td>
<td>Smart Cycler</td>
<td>MSSA/MRSA</td>
<td>Yes</td>
<td>$25.00</td>
</tr>
<tr>
<td>Cepheid</td>
<td>GenXpert</td>
<td>MSSA/MRSA</td>
<td>No</td>
<td>$50.00</td>
</tr>
<tr>
<td>Nanosphere</td>
<td>Verigene Reader</td>
<td>Gram positive, Gram negative bacteria</td>
<td>No</td>
<td>$50.00</td>
</tr>
<tr>
<td>BioFire</td>
<td>Film Array</td>
<td>Gram POS, NEG bacteria and Yeasts, and resistance markers</td>
<td>No</td>
<td>$120.00</td>
</tr>
</tbody>
</table>

Palavecino E. MRSA protocols 2nd Edition. 2013
Verification and Validation

CLIA Requirements

• **Verification**: Does the test work in my lab?
  • A one time process to confirm the test performance
  • Complexity and extent of verification varies by test

• **Validation**: Does the test still work?
  • A process to ensure that the test continues working as expected
  • QC, Proficiency testing, staff training and competency.
Impact of Molecular Testing on Patient Outcomes and Hospital Costs

Justifying Implementation

• Molecular testing often more expensive than traditional methods
• Full benefit should be analyzed in relation to patient care
• Integrate your clinical teams in the decision making and monitoring impact
Benefits of Rapid Viral Diagnosis Impact on Physician Decision Making

- Statistically significant - Better management of patients
  - Limit unnecessary antibiotic use
  - Limit unnecessary/increased appropriate antiviral use
  - Limit other laboratory testing/radiology – sepsis workup children
  - Manage high-risk patients
- Reduce hospital stay or time in the ER
- Other Benefits
  - Rapid outbreak identification of influenza
    - Prevent or limit community spread
  - Characterize epidemiology of influenza virus infections

Impact of Rapid Diagnosis Using PCR for Identification of MRSA/MSSA from Blood Cultures

- Implementation of RT-PCR for differentiation of MRSA and MSSA from BC with GPC
- Initial therapy was vancomycin
- Monitored changes to appropriate therapy pre-and-post rapid testing
- Mean time to switch from empiric vancomycin to cefazolin or nafcillin in patients with MSSA bacteremia was 1.7 days shorter post RT-PCT

Bauer KA et al. CID. 2010;51:1074-1080
### MRSA Screening Cost Savings

Estimated Effect on Unnecessary Contact Precaution Days Avoided and Costs Saved (with a single PCR)

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Passive cultures</th>
<th>Active surveillance cultures</th>
<th>PCR screening (1 Xpert MRSA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discontinuation rates of contact precautions</td>
<td>6.6%</td>
<td>26.2%</td>
<td>63.8%</td>
</tr>
<tr>
<td>Fewer contact precaution days</td>
<td>104</td>
<td>418</td>
<td>1841</td>
</tr>
<tr>
<td>Cost savings</td>
<td>$86,950</td>
<td>$349,472</td>
<td>$1,539,180</td>
</tr>
</tbody>
</table>

Shenoy et al, CID 2013 Jul;57(2):176-84
Rapid Detection of Pathogens in Positive Blood Cultures: Effects on Health Care Cost

Using Maldi-TOF

- Hospitalization cost reduction of $19,547/patient
- Estimated cost savings of ~18 million annually

Conclusions

- Early and accurate diagnosis of infections and appropriate antimicrobial therapy correlate with positive clinical outcomes

- Several molecular fully automated platforms are available for rapid diagnosis of infectious diseases and are becoming a useful tool in hospitals of all sizes

- It is challenging to implement rapid tests due to financial constraints and the difficulty of staffing the lab for frequent testing, but it is worthwhile due to decrease in LOS and costs.

- The microbiology laboratory needs the input of the antimicrobial stewardship committee and ID clinicians to prioritize the laboratory assays for implementation.