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
    - Obstetrics/Gynecology 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

**Complexity**

- Molecular Testing
  - 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 samples 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>BD</td>
<td>GBS, Influenza, C. difficile, CT/GC</td>
</tr>
<tr>
<td>BD Max</td>
<td>BD Max Smart Cycler, BD Max Viper, CT/GC</td>
</tr>
<tr>
<td>Gene-Probe</td>
<td>SmartCycler, SmartCycler, Viper, Panther or Tigris</td>
</tr>
<tr>
<td>Cepheid</td>
<td>GeneXpert, GeneXpert, GeneXpert, GeneXpert</td>
</tr>
<tr>
<td>Focus</td>
<td>3M Integrated cycler, 3M Integrated cycler</td>
</tr>
<tr>
<td>Meridian</td>
<td>Illumigene, Illumigene</td>
</tr>
<tr>
<td>Roche</td>
<td>Cobas 4800</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 trachomatis/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
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


Clinical Syndrome: Respiratory Infections

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 PlusPlus subtypes
   RSV/Influenza A/B/H1, H3, novel H1
   Also, ProFlu 1-8, ProFlu/MPV, ProFlu Adenovirus, On SmartCycler
5. Nanosphere Verigene
   RSV/Flu A/B/H1/H2
6. Focus – 3M
   RSV/Flu A/B & H1N1 2009
7. Cepheid, Xpert Flu A/B (A/H1N1 2009, H1, H3)

and more...

Multiplex PCR: Detection and differentiation of respiratory viruses

- NP swab
- throat swab
- multiplex

Film Array Respiratory Panel (BioFire) Detects 20 respiratory pathogens.
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 U.S. specially among those ≥65 years of age.

NNIS reports in AJIC (2000-2004)

Molecular Tests for Active Surveillance

- Detection of MRSA from Nasal Swab
  Appropriate collection of nasal swab is very important
  - Cepheid –GeneXpert: Fully automated (sample to result), Randox access
  - BD GeneOhm- Smart Cycler: Manual extraction, but automated amplification and detection
  - Smart Cycler
  - BD MAX: Fully automated
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</td>
<td>Becton Dickinson</td>
<td>Smart Cycler</td>
<td>92.0/94.6</td>
<td>2.5 hours</td>
</tr>
<tr>
<td>MRSA ACP</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</td>
<td>Becton Dickinson</td>
<td>BD MAX System</td>
<td>93.1/97.5</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</td>
<td>Roche Diagnostics</td>
<td>LightCycler</td>
<td>95.2/96.4</td>
<td>2 hours</td>
</tr>
<tr>
<td>Test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NucliSENS</td>
<td>Biomerieux</td>
<td>EasyQ System</td>
<td>95.8/96.8</td>
<td>3 hours</td>
</tr>
<tr>
<td>MRSA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EasyQ MRSA</td>
<td>Becton Dickinson</td>
<td>BD MAX System</td>
<td>93.1/97.5</td>
<td>2 hours</td>
</tr>
<tr>
<td>Xpert MRSA</td>
<td>Cepheid</td>
<td>GeneXpert</td>
<td>95.8/97.6</td>
<td>3 hours</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>tcd B</td>
<td>Manual</td>
<td>75-90 min</td>
<td>$25-$49</td>
</tr>
<tr>
<td>Xpert C Diff</td>
<td>tcd B</td>
<td>Automated</td>
<td>45 min</td>
<td>$45</td>
</tr>
<tr>
<td>Ilumigene</td>
<td>tcd A</td>
<td>Manual</td>
<td>70 min</td>
<td>$NA</td>
</tr>
<tr>
<td>Prodesse ProGastro</td>
<td>tcd B</td>
<td>Easy Mag</td>
<td>180 min</td>
<td>$25</td>
</tr>
</tbody>
</table>

Adapted from Carroll KC. Anaerobe 2011. 17: 170-174
Clinical Syndrome: Sepsis

- Mortality from sepsis range from 25% to 80%
- 1.7 million patients annually in the US
- ~ $14.8 billion spent on hospitalization annually
  
  CDC NCHS Data Brief. 2011
  

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

Survival according to treatment

Culture

Broad Spectrum

Targeted Treatment

Empiric Treatment

Targeted Treatment

Sepsis

Gram stain

Culture

Susceptibility

Multiplex PCR

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

Traditional Methods

Day 1

Empiric Treatment

Blood Spectrum

Targeted Treatment

Rapid Methods

Empiric Treatment

Targeted Treatment

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


Organisms Most Commonly Isolated from Blood Cultures

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

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>Staphylococcus aureus</td>
<td>mecA gene</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus lugdunensis</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus pneumoniae</td>
<td></td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td></td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>VanA and VanB</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td></td>
</tr>
<tr>
<td>Micrococcus spp.</td>
<td></td>
</tr>
<tr>
<td>Listeria spp.</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>S. aureus</td>
<td>Enterobacteriaceae (E. coli, K. pneumoniae, K. oxytoca, E. cloacae, Proteus, Salmonella) and KPC-production</td>
<td>Candida albicans</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>P. aeruginosa</td>
<td>Candida glabrata</td>
</tr>
<tr>
<td>(VRE/VE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus</td>
<td>A. baumannii</td>
<td>Candida lusitana</td>
</tr>
<tr>
<td>(Group A, B)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus</td>
<td>H. influenzae</td>
<td>Candida parapsilosis</td>
</tr>
<tr>
<td>pneumoniae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Listeria</td>
<td>N. meningitidis</td>
<td>Candida tropicalis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 hour per sample

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

<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 ceftazidim or nafcillin in patients with MSSA bacteremia was 1.7 days shorter post RT-PCT

Bauer K A 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>63.6%</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 Mati-TOF
- Hospitalization cost reduction of $19,647/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.