Why Use A Molecular Method?

- Need Speed
  - Rapid diagnosis leads to an intervention in the patient
  - Culture takes days to weeks
- Need Sensitivity
  - Down to a very few organisms
  - Non-culturable
- Need quantification, not just a qualitative result
- Need safety
- No other way to differentiate organism

Nucleic Acid Amplification Tests

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Don’t need live organism</td>
<td>Might detect dead organisms</td>
</tr>
<tr>
<td>Low limit of detection</td>
<td>Too sensitive-sometimes one is not</td>
</tr>
<tr>
<td></td>
<td>enough to be relevant.</td>
</tr>
<tr>
<td>Turn Around time</td>
<td>High cost-reagents, instrumentation</td>
</tr>
<tr>
<td>Safety of nucleic acid</td>
<td>Potential for cross-contamination</td>
</tr>
</tbody>
</table>

FDA Cleared or Approved Tests-Bacterial

- **C. difficile**
- **C. trachomatis**
  - NAA: considered gold standard/ standard of care
  - Up to 30% more sensitive than culture
  - Specificity approaching 100%
  - Broad range of acceptable specimen types
- **N. gonorrhoeae**
  - Standard of care is NAAT
  - Specificity improved over 1st generation tests
  - Broad range of specimen types
- Group A and Group B Strep
- MRSA/MSSA
  - PCR available for screening for carriage, screening to optimize patient management, quality measure for hospitals
- Mycobacteria species
- Vancomycin resistance
- Bio-threat agents
Gene Sequencing
- Use as a secondary approach when easy identification methods fail
- Instead of multiple biochemical test algorithms
- Less proficiency with some biochemical tests

Pros and Cons of Gene Sequencing

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relatively fast</td>
<td>Costly</td>
</tr>
<tr>
<td>Very specific</td>
<td>Equipment, Reagents, labor</td>
</tr>
<tr>
<td>Same protocol all isolates</td>
<td>Need pure isolate</td>
</tr>
<tr>
<td>Versus many biochemical assays</td>
<td>Database can fool you</td>
</tr>
<tr>
<td></td>
<td>Taxonomy and nomenclature issues</td>
</tr>
</tbody>
</table>

The Future of Molecular Diagnostics
- Molecular ID of positive blood cultures
- Increasing multiplex capabilities
  - Relevant pathogens within specimen type or clinical presentation
- Gene Sequencing
  - To remain the gold standard for isolate ID
  - Use for unusual organisms
- MALDI-TOF MS to replace conventional phenotypic identification for routine pathogens

Antimicrobial Stewardship: Focus on the Role of Microbiology
Roger L. White, PharmD, FCCP
South Carolina College of Pharmacy, MUSC

Defining Antimicrobial Stewardship
- Activity that includes the appropriate section, dosing route and duration of antimicrobial therapy
- Primary goal is to optimize clinical outcome while minimizing unintended consequences of antimicrobial use including:
  - Toxicity
  - Selection of pathogenic organisms
  - Emergence of resistance
- Secondary goal-reduce health care costs without adverse impact on quality of care
  - Delite et al. Clin Infect Dis. 2007;44:159-77

Antimicrobial Stewardship Team
- ID physician
- Clinical Pharmacists with ID training
- Clinical Microbiologist
• Information system specialist
• Infection control professional
• Hospital epidemiologist (optional)

What are the Elements of Antimicrobial Stewardship
• Core Strategy 1: Prospective Audit with Intervention and Feedback
  o Concurrent review of patients receiving antimicrobials
  o Inappropriate orders initiate interaction between antimicrobial members and the prescriber

• Core Strategy 2: Formulary Restriction/Preauthorization
  o Effective method to control antibiotic use and cost

Microbiology Role In Stewardship
• Detection and identification (rapid) of organisms
• Susceptibility testing in individual patients
  o Reporting procedures (cascade reporting)
    ▪ IF the microbe is susceptible to X don’t report Y
  o Reporting procedures (Hiding susceptibilities)
    ▪ Don’t report drugs you don’t want prescribers to sue (e.g. fluoroquinolones for Staphylococcus aureus bacteremia)
  o Reporting procedures (notes on reports)
    ▪ Helpful notes attached to culture reports
    ▪ Link to guidelines
    ▪ Help with interpreting cultures
• Breakpoints interpretation/implementation
• Antibiogram preparation and interpretation
  o Preparation and analysis of cumulative susceptibility testing at least annually
    ▪ CLSI Doc M39-A provides guideline for preparation
      • Include only species with at least 30 isolates
      • Include diagnostic (not surveillance) isolates
      • Include 1st isolate/patient
      • Include only drugs routinely tested
      • Calculate %S (do not include %I)
• Evaluation of resistance trends

Why doesn’t every institution have an antimicrobial stewardship team?
• Funding Issues
  o Limited resources available
    ▪ May computer with other programs (e.g. infection control)
  o Overall antimicrobial stewardship program costs are not well-described in most studies
  o Proof of sustained benefit is lacking for most interventions
• Implementation Issues
  o Lack of direction in the guidelines for smaller, non-academic institutions
Guidelines don’t address outpatient or long term care facilities

Fear of being labeled “Antibiotic Police”

Programs may be perceived as self-serving (endorsed by those with the most gain)

No endorsement by QA groups or accreditation organizations

New Guidelines for the Prevention of Early-Onset Group B Streptococci Disease

What’s A Lab To Do?

Roberta B. Carey Ph.D.

Center for Disease Control and Prevention

2010 Guidelines: Collection and Transport

- Type of swab acceptable for antenatal screening vaginal/rectal swabs only; cervical swabs or perianal/perirectal not acceptable
- Recommend use of non-nutritive transport media-e.g. Amies or Stuart’s (with or without charcoal)
- Group B Streptococcus (GBS) viability declines over 1-4 days at high temperatures, refrigerate if dealy before processing

2010 Guidelines: Culture

- Remove swab from transport media and inoculate selective broth medium
  - TransVag broth
    - Incubate 18-24 hours at 35-37 C and subculture to sheep blood agar (SBA), Columbia agar with colistin/Nalidixic acid (CNA )or chromagar
  - LIM broth
    - Incubate 18-24 hours at 35-37 C and subculture to BAP,CNA or chromagar
  - Selective enrichment broth
    - Incorporates chromogenic pigments for the detection of GBS using color detection
    - Examples: Strep B carrot broth or Granada Biphasic broth
    - Monitor for color change
- Chromogenic Media
  - Studies show majority agars and broth equal to or better than SBA/CNA and LIM broth for GBS recovery
    - Added advantage of detection within 24 hours
    - Positives do not require confirmation by latex agglutination
- Non- hemolytic GBS
  - Approximately 4% of invasive GBS isolates are non-0hemolytic
  - Chromogenic broths do not detect non-hemolytic GBS
  - Most chromogenic agars do not detect non-hemolytic GBS
- Need to subculture all negative chromogenic media if they don’t detect non-hemolytic isolates
- Optional Direct Broth Testing
  - Detection of GBS can be determined directly from broth media using latex agglutination, probes or nucleic acid amplification tests (NAAT) such as PCR.

### 2010 Guidelines: Nucleic Acid Amplification Tests
- Recommendation for PCR antepartum testing (antepartum before labor begins)
  - For identification of GBS from enrichment broth
  - Not to replace culture
- Recommendations for PCR for intrapartum (woman in labor)
  - NAAT may be performed on patients with unknown GBS status and no risk factors who present at triage or labor/delivery
  - For clinical utility test results should be reported within 2 hours and PCR testing should be available 24/7

<table>
<thead>
<tr>
<th>PCR Assays Available</th>
<th>GeneOhm</th>
<th>Smart GBS</th>
<th>GeneXpert</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hands on time</td>
<td>18 min</td>
<td>25 min</td>
<td>2-3 min</td>
</tr>
<tr>
<td>Total Test Time</td>
<td>70 min</td>
<td>75 min</td>
<td>≤ 80 min</td>
</tr>
<tr>
<td>Complexity</td>
<td>High</td>
<td>High</td>
<td>Moderate</td>
</tr>
<tr>
<td>External Controls</td>
<td>yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cost</td>
<td>$18-26</td>
<td>$18-26</td>
<td>$40-45</td>
</tr>
</tbody>
</table>

### PCR Tests Performance
Based on Manufacturing Package Insert

<table>
<thead>
<tr>
<th>Test</th>
<th>% Sensitivity</th>
<th>% Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD GeneOhm Strep B</td>
<td>94%</td>
<td>96%</td>
</tr>
<tr>
<td>Cepheid Smart GBS</td>
<td>85%</td>
<td>97%</td>
</tr>
<tr>
<td>Cepheid Xpert GBS</td>
<td>92%</td>
<td>90%</td>
</tr>
</tbody>
</table>

### 2010 Guidelines: GBS Bacteriuria
• GBS can cause symptomatic and asymptomatic UTI
• GBS bacteriuria is a marker for heavy genital tract colonization and these women should receive IAP
• Laboratories should report significant concentrations ($\geq 10^4$ cfu/ml) of GBS bacteriuria in pregnant women
• Report $\geq 10^4$ GBS in pure culture or in the presence of a second organism (E.coli and GBS)

2010 Guidelines: Antimicrobial Susceptibility Testing (AST)
• Specimen requisitions for GBS testing should identify the patient at high risk for anaphylaxis as penicillin allergic and antibiotic susceptibility testing for clindamycin and erythromycin should be ordered
• CLSI M100-S21 performance Standards recommend using
  o Disk diffusion
  o Broth microdilution
    ▪ FDA-cleared/approved commercial system may also be used
  o Testing for inducible clindamycin resistance
    ▪ D Zone of other validated test

Summary of Key Changes
• Vaginal/Rectal Culture
  o Expand swab options
  o Option to use chromogenic broths and agars
  o Option to use antigen detection, probe NAAT from broth
  o Direct plating can accompany broth inoculation (not replace)
• Molecular ID Tests
  o Not recommended for routine 36-37 week antenatal screening
  o Recommended direct testing for women with no risk factors and no prenatal screening
  o Recommended for use on broth enriched cultures to ID GBS
• Urine Culture Screening
  o Report GBS at $\geq 10^4$ CFU/ml in pregnant women
• AST
  o Test for inducible clindamycin resistance when women is at high risk for anaphylaxis due to penicillin allergy
CDC Website For GBS: http://www.cdc.gov/groupb