The Clinical Microbiology Laboratory of the 21st Century

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Disclosures

- **Grants**: CD Diagnostics, Merck, Contrafect, Hutchison Biofilm Medical Solutions, Accelerate Diagnostics, Contrafect, Shionogi; monies paid to Mayo Clinic

- **Consultant**: Curetis, Specific Technologies, Next Gen Diagnostics, Selux Dx, GenMark Diagnostics, PathoQuest, Heraeus Medical, Qvella; monies paid to Mayo Clinic

- **Patents**: *Bordetella pertussis/parapertussis* PCR issued, device/method for sonication with royalties paid by Samsung to Mayo Clinic, anti-biofilm substance issued

- **Editor’s stipend**: ASM and IDSA

- **Honoraria**: NBME, Up-to-Date, the Infectious Diseases Board Review Course
Objectives

• Review the revolution in clinical microbial diagnostics over the past decade

• Learn about application of advances in microbial diagnostics in clinical practice

• Appreciate the science needed to inform the use of diagnostics in addressing antibacterial resistance
70% Clinical Decisions Substantially Based on Results of Diagnostic Tests

- Laboratory costs account for ~4% of health care costs; laboratory tests - single highest volume medical activity - # tests doubled in 20 years (~3,500)
- Costs rising 15-25%/year - faster than other areas of medicine - mostly due to molecular tests
- ~45% laboratory tests underutilized
- ~21% laboratory tests unnecessary

Outline

1. Proteomics
2. POC nucleic acid amplification tests
3. Panel-based molecular diagnostics
4. Laboratory automation
5. Sequencing-based diagnostics
6. Susceptibility testing improvements
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The main application of proteomics in clinical microbiology is

A. Serologic testing
B. Identification of cultured bacteria and fungi
C. Antimicrobial susceptibility testing
D. Viral confirmation in cell lines
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20 Minute POC Organism-Specific PCR Cobas® Liat (Roche)

- Influenza A/B ± RSV, *Streptococcus pyogenes*
- Swab used to collect specimen → placed in liquid medium
- Liquid pipetted into reaction container
- Barcode scanned
- Reaction container placed into instrument

8-15 Minute POC Organism-Specific NAAT
Alere™ i Influenza A & B, Alere™ i RSV, Alere™ i Strep A
NEAR technology (Nicking Enzyme Amplification Reaction)
Changing Diagnostic Paradigms for Microbiology

A report from the American Academy of Microbiology and the American Society for Microbiology
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1. Proteomics
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Blood Culture Bottle Panels
Randomized Controlled Clinical Trial
Mayo Clinic 2013-2014

Patients with positive blood cultures
Stratified randomization (age, ICU, transplant service)

CONTROL

- Gram stain called to service
- Standard subculture and susceptibility (1-3 d)

RAPID TEST ALONE

- Gram stain called to service
- Standard subculture and susceptibility (1-3 d)
- Rapid test plus lab call with comments (1 h)

RAPID TEST/STEWARDSHIP

- Gram stain called to service
- Standard subculture and susceptibility (1-3 d)
- Rapid test plus lab call with comments (1 h)
- ID MD/pharmacist call with specific treatment recommendations


Supported by the National Institute of Allergy And Infectious Diseases of the National Institutes of Health under Award Number UM1AI104681 (Antibacterial Resistance Leadership Group)
# FilmArray® Blood Culture Identification Panel (BioFire)

<table>
<thead>
<tr>
<th>Gram Positive Bacteria</th>
<th>Gram Negative Bacteria</th>
<th>Fungi</th>
<th>Resistance Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus</td>
<td>Klebsiella oxytoca</td>
<td>Candida albicans</td>
<td>bla&lt;sub&gt;KPC&lt;/sub&gt;</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Klebsiella pneumoniae</td>
<td>Candida glabrata</td>
<td>mecA</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>Serratia</td>
<td>Candida krusei</td>
<td>vanA/vanB</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>Proteus</td>
<td>Candida parapsilosis</td>
<td></td>
</tr>
<tr>
<td>Enterococcus Listeria monocytogenes complex</td>
<td>Acinetobacter baumannii</td>
<td>Candida tropicalis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Haemophilus influenzae</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neisseria meningitidis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enterobacteriaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Escherichia coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enterobacter cloacae</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Clinical Outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Control (n=207)</th>
<th>Rapid Test (n=198)</th>
<th>Rapid Test + Stewardship (n=212)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of stay (days)</td>
<td>8 (5,15)</td>
<td>8 (5,15)</td>
<td>8 (5,16)</td>
<td>0.60</td>
</tr>
<tr>
<td>30-day mortality</td>
<td>22 (10.6%)</td>
<td>20 (10.1%)</td>
<td>18 (8.5%)</td>
<td>0.74</td>
</tr>
<tr>
<td>30-day readmission w/same organism</td>
<td>6 (2.9%)</td>
<td>6 (3%)</td>
<td>8 (3.8%)</td>
<td>0.88</td>
</tr>
<tr>
<td>Toxicity/adverse drug reaction</td>
<td>3 (1.4%)</td>
<td>3 (1.5%)</td>
<td>2 (0.9%)</td>
<td>0.82</td>
</tr>
<tr>
<td>Blood culture clearance in 3d</td>
<td>147 (71%)</td>
<td>131 (66.2%)</td>
<td>146 (68.9%)</td>
<td>0.79</td>
</tr>
<tr>
<td><em>C. difficile</em> /Drug-resistant organism(^1) within 30d</td>
<td>15 (7.2%)</td>
<td>16 (8.1%)</td>
<td>21 (9.9%)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

\(^1\)VRE, MRSA, ESBLs, Gram negative bacilli resistant to ≥3 drug classes

## Comparison of Time To Identification, Susceptibility Results, and Antibiotic Modifications

<table>
<thead>
<tr>
<th>Timeline, hours (h)</th>
<th>0</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>48</th>
<th>60</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=169)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid test (n=147)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid test + Stewardship (n=165)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **ID**: Organism identification
- **AST**: Phenotypic antimicrobial susceptibility report
- **D**: De-escalation
- **E**: Escalation

*Significant vs. control; †Significant vs. control and rapid multiplex PCR alone

**Antimicrobial stewardship oversight in second intervention group**

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# Automated Specimen Processing

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Specimen type</th>
<th>Inoculation technique</th>
<th>Capacity (plates inoculated/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Innova</strong>&lt;br&gt;BD</td>
<td>Liquid based specimen</td>
<td>Loop</td>
<td>180</td>
</tr>
<tr>
<td><strong>InoqulA FA/MI</strong>&lt;br&gt;(Full Automation/Manual Interaction)&lt;br&gt;BD-Kiestra</td>
<td>Liquid based specimen (FA)&lt;br&gt;Swab (MI)</td>
<td>Bead</td>
<td>400</td>
</tr>
<tr>
<td><strong>PREVI Isola</strong>&lt;br&gt;bioMérieux</td>
<td>Liquid based specimen</td>
<td>Comb</td>
<td>180</td>
</tr>
<tr>
<td><strong>WASP™</strong>&lt;br&gt;(Walk away specimen processor)&lt;br&gt;Copan</td>
<td>Liquid based specimen</td>
<td>Loop</td>
<td>180</td>
</tr>
</tbody>
</table>
Total Laboratory Automation

Wasp Lab

BD-Kiestra
Digital Imaging
Virtuo Blood Culture System (bioMérieux)
Outline

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The 16S ribosomal RNA (rRNA) gene is a conserved gene among

A. Fungi
B. Viruses
C. Parasites
D. Bacteria
E. Prions
16S Ribosomal RNA Gene
Mycobacterium lepromatosis
MLST

- 5-7 housekeeping genes
  - Sequence type (ST) and Clonal complex (CC)
  - Public nomenclature

Used mainly for studying bacterial phylogeny & evolution of population lineages

Core Genome MLST

- Hundreds/thousands of ‘core genome’ genes
  - Scalable, portable and understandable
  - Public, additive, and expandable nomenclature
  - Higher discrimination power than MLST

MLST

- 7 genes
- 0.1% of FAM18 genome

Core Genome MLST

- 1,241 genes
- 54.5% of FAM18 genome
NICU MRSA Outbreak(s)

- Reinforcement of basic practices
- Reinforcement of basic practices
- Reinforcement of basic practices
- Reinforcement of basic practices
- Environmental sampling and deep cleaning
- Observe practices
- Environmental remodeling

- Targeted screening for NICU infants
- Universal weekly screening for NICU infants

Number of cases

- Screening isolate (infant)
- Screening isolate (HCW)
- Clinical isolate (infant)

Madigan et al. Infect Cont Hosp Epi 2018;39:1412-18
Neonatal Intensive Care Unit (NICU) Isolates (Clinical & Surveillance) Neonates & Healthcare Workers
Outbreak Timeline with MRSA Cases Shown by WGS Group

Madigan et al. Infect Cont Hosp Epi 2018;39:1412-18
Metagenomic Shotgun Whole Genome Sequencing

Sequence ALL DNA present using short 150-300 bp reads
Current Orthopedic Implant Processing - Mayo Clinic

- Prosthesis Placed in Container (Operating Room)
- 400 ml Ringer’s Solution Added
- Vortex 30 sec
- Vortex 30 sec
- Centrifuge 5 min
- Aspiration
- Plating
- Sonicate 5 min
- Vortex 30 sec

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Methods

- Microbial DNA enrichment: MoLYsis Basic5 kit
- DNA extraction: MoBio Bacteremia DNA isolation kit
- Whole genome amplification: Qiagen REPLI-g Single Cell kit
- Amplified DNA purification: Agencourt Ampure XP beads
- Paired-end library prep: NEBNext Ultra DNA Library Prep Kit
- Sequencing: Illumina HiSeq 2500 in rapid run mode with paired end reads at 250 cycles (multiplexed 6 samples/lane, ~30,000,000 paired-end reads/sample)
- Adapter sequence removal: Trimmomatic (v0.36)
- Human & PhiX sequence removal: BioBloom tools (v2.0.12)
- Data analysis: Livermore Metagenomics Analysis Toolkit (LMAT, v1.2.6) and MetaPhIAn2
Metagenomics versus Culture

- 408 sonicate fluid samples tested
  - 195 aseptic failures
  - 213 PJIs

### Metagenomic Analysis vs. Sonicate Fluid Culture

<table>
<thead>
<tr>
<th></th>
<th>Samples</th>
<th>Identical Findings</th>
<th>Organisms Not Identified by Metagenomics</th>
<th>New Organisms Detected by Metagenomics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aseptic Failures</strong></td>
<td>195</td>
<td>188 (96.4%)</td>
<td>N/A</td>
<td>7 (3.6%)</td>
</tr>
<tr>
<td><strong>Culture-Positive PJIs</strong></td>
<td>115</td>
<td>99 (86.1%)</td>
<td>6 (5.2%)</td>
<td>11 (9.6%)</td>
</tr>
<tr>
<td><strong>Culture-Negative PJIs</strong></td>
<td>98</td>
<td>55 (56.1%)</td>
<td>N/A</td>
<td>43 (43.9%)</td>
</tr>
</tbody>
</table>

New or Missed Identifications by Metagenomics vs. Sonicate Fluid Culture

| PJI Organisms Not Detected by Metagenomics | Bacillus species | Mycobacterium abscessus Porphyromonas species | Pseudomonas aeruginosa (2) |
| New Organisms Detected in Aseptic Failures | Cutibacterium acnes (2) | Staphylococcus aureus (3) | Streptococcus sanguinis (2) |
| New Organisms Detected in Culture-Positive PJIs | Anaerococcus obesiensis Clostridium species Cutibacterium acnes Enterobacter cloacae* | Finegoldia magna (3)* Peptoniphilus harei Prevotella nanciensis Staphylococcus aureus | Staphylococcus epidermidis (6) Staphylococcus lugdunensis (2) Varibaculum cambriense |
| New Organisms Detected in Culture-Negative PJIs | Anaerococcus urinae Candida albicans (2)* Candida parapsilosis* Clostridium perfringens Corynebacterium pseudogenitalium Cutibacterium acnes Enterococcus faecalis (3)* | Enterobacter cloacae (2)* Facklamia languida Granulicatella adiacens (2)* Mycobacterium bovis BCG* Mycoplasma salivarium Peptoniphilus species Pasteurella multocida* | Staphylococcus aureus (10)* Staphylococcus epidermidis (5)* Staphylococcus haemolyticus (2)* Staphylococcus lugdunensis Streptococcus agalactiae (4) Streptococcus dysgalactiae (4)* Streptococcus oralis* |

53 yo Man – Right Knee PJI

A.

R knee arthroplasty

Knee pain, stiffness, swelling, and sinus tract development

Multiple courses of oral antibiotics

Referral to Mayo Clinic for evaluation

6 weeks IV antibiotics

R knee arthroplasty resection

Sinus tract development

Nov 2012

April 2013

2014

July 2015

Feb 2016

April 2016

July 2016

April 2017

Synovial fluid: 28,756 cells/mm³ (93% PMNs), cultures negative. ESR: 53, CRP: 71.6

Synovial fluid: 2,288 cells/mm³ (80% PMNs), cultures negative. PCR for *M. hominis* negative. ESR: 44, CRP: 51.3

ESR: 44, CRP: 51.3

Metagenomic analysis of Feb 2016 sonicate fluid positive for *M. salivarum*

Synovial fluid: 11,596 cells/mm³ (94% PMNs). 16S rRNA gene PCR/sequencing positive for *M. salivarum*

B.

All Reads (27,984,652)

Microbial Reads (1906)

Bacterial Reads (1881)

- Human (27,049,593)
- Read Too Short (87,868)
- Low LMAT Score (812,657)
- Microbial (1906)
- No Database Hits (16,796)
- Chimeras (420)
- Cellular Organism (15,412)

- Bacteria (1,881)
- Protozoa (14)
- Fungi (11)
- Viruses (0)

- Mycoplasma (1,796)
- Curvibacter (2)
- Acinetobacter (58)
- Burkhholderia (1)
- Propionibacterium (19)
- Rubrivivax (1)
- Paenibacillus (4)
Antibiotic Resistance Prediction

Macrolide resistance-associated mutations in 23S rRNA gene of *M. pneumoniae*


Alignment of case to reference *M. salivarium* and *M. pneumoniae* 23S rRNA genes

Metagenomic Shotgun Sequencing of Synovial Fluid

<table>
<thead>
<tr>
<th>Samples</th>
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<th>Organisms Not Identified by Metagenomics</th>
<th>New Organisms Detected by Metagenomics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aseptic Failures</td>
<td>61</td>
<td>56 (91.8%)</td>
<td>1 (1.6%)</td>
</tr>
<tr>
<td>Synovial Fluid Culture-Positive PJIs</td>
<td>82</td>
<td>67 (81.7%)</td>
<td>14 (17.1%)</td>
</tr>
<tr>
<td>Synovial Fluid Culture-Negative PJIs</td>
<td>25</td>
<td>21 (84.0%)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

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Rapid Phenotypic Susceptibility Testing –
Example, Accelerate ID/AST (Application –
Positive Blood Culture Bottles)
Sample Prep - Gel Electrofiltration

- Blood cells lysed
- Sample added to gel electrofiltration well (contains gel with pores smaller than bacteria)
- Positive charge applied → debris migrates into gel leaving bacteria behind.
- Negative charge applied → bacteria move to center of well for ease of retrieval.
Electrokinetic Concentration

TIME-LAPSE IMAGE OF SURFACE CAPTURE IN LESS THAN 5 MINUTES
Antimicrobial Susceptibility Testing

- Time-lapse imaging and analysis of bacterial growth
- Individual bacterial response to single antibiotic concentration over time

*E. coli* vs. 4 μg/mL piperacillin-tazobactam

![Graph showing bacterial growth over time](image)
Multicenter Study
Accelerate Pheno™ System

- Fresh clinical and seeded blood cultures
- VITEK® 2 identification, broth microdilution or disk AST
- Identification sensitivities 94.6-100%

Gram-positive cocci
- Essential agreement 97.6%
- Categorical agreement 97.9%
- Very major, major & minor error rates: 1.0%, 0.7% & 1.3%

Gram-negative bacilli
- Essential agreement 95.4%
- Categorical agreement 94.3%
- Very major, major & minor error rates: 0.5%, 0.9% & 4.8%

Pancholi et al. J Clin Microbiol. 2018;56i:e01329-17
Rapid Identification and Susceptibility Testing for Gram-Negative Bacteremia

- Multi-center, prospective, randomized, controlled, factorial design trial evaluating antimicrobial utilization, clinical outcomes, and healthcare costs among patients with BSIs caused by GNB who receive:
  - Standard culture and AST plus bacteremia-focused antimicrobial stewardship program oversight
  - Rapid identification and AST with bacteremia-focused antimicrobial stewardship program oversight
More Reflections…

• NEED actionable tests which when acted on, improve patient outcomes

• Ideal tests remain to be defined
  • …and then commercialized
  • Who will commercialize “niche” tests?
    ➢ e.g., Abbott IRIDICA

• Ultimately, tests need to be paid for
  • What if more testing shown to be beneficial?
  • What if novel testing adds cost
Summary

• Proteomics prevail (for colony picking)
• PCR moves to POC
• Panels proliferate
• Automation advances
  • Specimen plating, plate incubation, plate reading & work-up
• Sequencing sprawls
  • Bacterial whole genome sequencing – typing Gold standard
  • 16S rRNA gene PCR/sequencing and metagenomic shotgun sequencing for pathogen detection
    • Clinically-infected but culture-negative
• Susceptibility speeds
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