Rapid Bacterial Diagnostics
Are We There Yet?

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Disclosures

- Bacterioscan – Advisory Board
- Cubist – Speakers Bureau, in-house training
- Merck – Speakers Bureau
- Cepheid – Collaborative Studies
Objectives

- Define “Rapid”, “We” and “There”
- Old technology – Don’t throw out the baby with the bath water
- New technology – Keeping me from retirement
- Are we there yet?
Rapid

Minutes to 3 hours
Who are “WE”

- Clinical Microbiology Laboratory
- Physician
  - Admit/treat/do not treat
- Pharmacy/antibiotic stewardship
- Infection Control
- Team approach: all of the above
  - e.g., ID, pharmacy, lab, IC, etc.
- Enrollment in clinical study
Team Approach

• No result sitting in a lab by itself is useful
• In order for RBD to work, need a systems approach
• Who calls who with what?
• What is the desired intervention?
• How does it effect outcome?
• How best use results to enroll or not
What is “There”

- A single pathogen
- Any pathogen
- Multiple pathogens
- Anatomical specific pathogens
- Antibiotic specific result: S or R
Desired Outcome(s)

- Reduce time to appropriate therapy
- Reduce length of stay
- Reduce transmission of pathogen
- Reduce cost
- Enroll patient is a clinical trial or not
## Goals

<table>
<thead>
<tr>
<th>Industry</th>
<th>Micro/ID/Pharmacy/IC</th>
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<tbody>
<tr>
<td>Increase clinical trial efficiency</td>
<td>Reduce time to appropriate treatment Directed rather than empirical therapy</td>
</tr>
<tr>
<td>Increase likelihood of enrollment based on rapid pathogen detection</td>
<td>Improve patient care/reduce length of stay</td>
</tr>
<tr>
<td>Reduce patients who have to be excluded/dropped because of no or wrong pathogen(s)/ not susceptible</td>
<td>Decrease emergence of antibiotic resistance/antibiotic stewardship</td>
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</table>
Rapid Diagnosis
Directly From Specimens
Old Technology
Gram
Stains
Gram Stains Can Work

- To make a Gram stain work requires work
  - Appropriate specimens
    - Sputum not spit
    - Aspirate not swab
- Selection within specimen
  - Sputum is heterogeneous
  - Select mucous plugs
Gram stains predicted community respiratory pathogens in AECBB study patients with high accuracy and PPV

Mucous plugs selected, examined for neutrophils and if present, slide gram stained. If GS showed a predominant organism, the other half of the mucous plug was used for culture
Predicting Pathogens in CA-AECBB

• 480 patients at study entry
  - GS predicted the cultured pathogen 321 times (67%)
  - Predicted 2 pathogens but grew only 1 73 times (15%)
  - Predicted 2 pathogens and grew 2 pathogens 35 times (7%)
  - Predicted 1 or 2 pathogens and grew 1 predicted and 1 not predicted 38 times (8%)
  - Predicted a pathogen and a pathogen not grown 13 times (3%)
  - Also predicted absence of pathogen

Expectorated Sputa
Predicting the Pathogen in Suctioned Respiratory Secretions
The Holy Grail
Rapid Bacterial Diagnosis
Directly From Blood
Limitations to the Rapid Detection of Bacteria in Blood

• Very Few Targets
  – Most bacteremic patients have very few bacteria/ml of blood
  – To diagnose bacteremia we take 20 ml of blood \( \times 2 \) and put 10 ml in each of 4 bottles
  – Positive patients often have only 1 or 2 positive bottles
  – Takes 1-5 days to get results
**Procalcitonin Sepsis Meta – Analysis**

- Protein produced by numerous cells/organs in response to inflammation due to bacterial infections (+ other things)
- 30 studies, 3244 patients with sepsis
  - Mean sensitivity = 77%
  - Mean specificity = 79%
- Useful in dx of severe sepsis but results have to be interpreted with caution

T2 Magnetic Resonance Enables Nanoparticle-Mediated Rapid Detection of Candidemia in Whole Blood

Lori A. Neely,¹ Mark Audeh,¹ Nu Ai Phung,¹ Michael Min,¹ Adam Suchocki,¹ Daniella Plourde,¹ Matthew Blanco,¹ Vasiliki Demas,¹ Lynell R. Skewis,¹ Theodora Anagnostou,² Jeffrey J. Coleman,²,³ Parris Wellman,¹ Eletherios Mylonakis,²,³ Thomas J. Lowery¹

Candida spp. cause both local and disseminated infections in immunocompromised patients. Bloodstream infections of Candida spp., known as “candidemia,” are associated with a high mortality rate (40%), which is mainly attributed to the long diagnostic time required by blood culture. We introduce a diagnostic platform based on T2 magnetic resonance (T2MR), which is capable of sensitive and rapid detection of fungal targets in whole blood. In our approach, blood-compatible polymerase chain reaction is followed by hybridization of the amplified pathogen DNA to capture probe–decorated nanoparticles. Hybridization yields nanoparticle microclusters that cause large changes in the sample’s T2MR signal. With this T2MR-based method, Candida spp. can be detected directly in whole blood, thus eliminating the need for analyte purification. Using a small, portable T2MR detection device, we were able to rapidly, accurately, and reproducibly detect five Candida species within human whole blood with a limit of detection of 1 colony-forming unit/ml and a time to result of <3 hours. Spiked blood samples showed 98% positive agreement and 100% negative agreement between T2MR and blood culture. Additionally, performance of the assay was evaluated on 21 blinded clinical specimens collected serially. This study shows that the nanoparticle- and T2MR-based detection method is rapid and amenable to automation and offers clinicians the opportunity to detect and identify multiple human pathogens within hours of sample collection.
Assay Design

A
~2 ml blood sample → Blood cell lysis & Candida cell concentration → Remove supernatant → Lyse Candida cells → PCR lysate → Aliquot & hybridize with particles → T2 detection

B
Target complementary capture probe A → Add sample (i.e. blood containing target DNA) → DNA target hybridizes to capture probes forming inter-particle linkages: a change in T2 measured as agglomeration ensues
T2MR Direct Detection

- No background interference eliminates sample preparation and extraction of targets
- No manipulation or extraction of the target analyte enables superior specificity and sensitivity
- Measuring the magnetic properties of the entire water population and not just the target provides breakthrough sensitivity in dirty samples

**T2Candida – Critical Performance Metrics**

- **Limit of Detection (LoD) as low as 1 CFU/mL**
- **Anti-fungals in a patient sample can prohibit cell growth in blood culture, leading to a false negative result**
- **Equivalent or better sensitivity than blood culture with 25x faster turn-around time**

### Target Species | LoD
---|---
C. albicans | 3 CFU/mL
C. tropicalis | 3 CFU/mL
C. parapsilosis | 1 CFU/mL
C. glabrata | 2 CFU/mL
C. krusei | 2 CFU/mL

2. Beyda, M. Jahangir Alam, Kevin W. Garey, *Diagnostic Microbiology & Infectious Disease* 2013 in press.
Rapid Bacterial and Viral Diagnosis
Multi-Plex PCR
FilmArray

- Two minutes of hands-on time
- Results in about 1 hour
- 20 viral and bacterial pathogens
- Closed System – Contamination is not an issue
- PCR based Molecular
Respiratory Panel  FDA Cleared

**Viral**

Adenovirus
Coronavirus 229E
Coronavirus HKU1
Coronavirus OC43
Coronavirus NL63
Human Metapneumovirus
Human Rhinovirus/Enterovirus
Influenza A
Influenza A/H1
Influenza A/H1-2009
Influenza A/H3
Influenza B

**Parainfluenza**

Parainfluenza 1
Parainfluenza 2
Parainfluenza 3
Parainfluenza 4
RSV

**Bacterial**

*Bordetella pertussis*
*Chlamydophila pneumoniae*
*Mycoplasma pneumoniae*

* FDA-Cleared for the first time
The Evidence

- Overall, 95% sensitivity and 99% specificity

### Clinical Sensitivity and Specificity of the FilmArray Respiratory Pouch

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prospective</td>
<td>Retrospective</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>88.9%</td>
<td>100%</td>
</tr>
<tr>
<td>Coronavirus HKU1</td>
<td>95.8%</td>
<td>n/a</td>
</tr>
<tr>
<td>Coronavirus NL63</td>
<td>95.8%</td>
<td>n/a</td>
</tr>
<tr>
<td>Coronavirus 229E</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Coronavirus OC43</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Human Metapneumovirus</td>
<td>94.6%</td>
<td>n/a</td>
</tr>
<tr>
<td>Human Rhinovirus/Enterovirus</td>
<td>92.7%</td>
<td>95.7%</td>
</tr>
<tr>
<td>Influenza A</td>
<td>90.0%</td>
<td>n/a</td>
</tr>
<tr>
<td>Influenza A/H1</td>
<td>n/a</td>
<td>100%</td>
</tr>
<tr>
<td>Influenza A/H3</td>
<td>n/a</td>
<td>100%</td>
</tr>
<tr>
<td>Influenza A/H1-2009</td>
<td>88.9%*</td>
<td>100%</td>
</tr>
<tr>
<td>Influenza B</td>
<td>n/a</td>
<td>100%</td>
</tr>
<tr>
<td>Parainfluenza Virus 1</td>
<td>100%*</td>
<td>97.1%</td>
</tr>
<tr>
<td>Parainfluenza Virus 2</td>
<td>87.4%*</td>
<td>100%</td>
</tr>
<tr>
<td>Parainfluenza Virus 3</td>
<td>95.8%</td>
<td>100%</td>
</tr>
<tr>
<td>Parainfluenza Virus 4</td>
<td>100%*</td>
<td>100%</td>
</tr>
<tr>
<td>Respiratory Syncytial Virus</td>
<td>100%</td>
<td>n/a</td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>100%*</td>
<td>94.6%</td>
</tr>
<tr>
<td>Chlamydia pneumoniae</td>
<td>100%*</td>
<td>100%</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>100%*</td>
<td>84.4%</td>
</tr>
</tbody>
</table>

*Spiked Chlamydia pneumoniae samples were used to test retrospective sensitivity.

*Due to low prevalence in the prospective study, clinical sensitivity for these pathogens was based on less than 10 positive samples.
After all panels are FDA-cleared, FilmArray will have assays covering 125 of the most common pathogens that cause death and disease.
Rapid Bacterial Diagnosis Directly From Urine
Narrow Angle Laser Forward Scatter Measurement

- Measurement of scattered light intensity and motion
- Particle size of interest: 0.2 to 10 microns
- Analogous to OD measurement - optimized for very low concentrations
- Samples held at 37° C to promote organism growth
- Automated for real-time observation of growth over minutes, hours, days
Rapid AST Using Laser Microbial Growth Monitor

- Laser Scatter Measurement with onboard incubation of up to 24 samples at a time
- Five logs of linear dynamic range for quantitation of bacteria from $\sim 10^4$ to $\sim 10^9$ CFU/mL in clear fluids
- Available early 2014

### UTI Detection Performance

- **Fast Positive Detection (10 minutes)**
  - Sensitivity: 87.5%
  - Specificity: 87.9%
  - PPV: 51.9%
  - NPV: 97.9%

- **Elimination of Negatives (90 minutes)**
  - Sensitivity: 96.9%
  - Specificity: 85.1%
  - PPV: 49.2%
  - NPV: 99.5%

Clinical Study conducted with St Louis University Hospital Jan-May 2013 in 248 patients with 14.6% UTI positive at $>1 \times 10^4$ CFU/ml, in matched pairings of preserved and unpreserved/refrigerated specimens, tested 24 hours after collection.
Rapid Bacterial Diagnosis
From Colonies or Broth
QuickFISH/PNA FISH
Positive Blood Cultures

- **QuickFISH**: Gram-Negative Bacilli (20 minutes)
  - *E. coli*, *K. pneumoniae*, or *P. aeruginosa*
- **QuickFISH**: Gram-Positive Cocci (20 minutes)
  - *S. aureus/CNS*
  - *E. faecalis/E. faecium*
- PNA FISH for Candida (**QuickFISH coming**)

*QuickFISH* is a trademark of AdvanDx
Blood Culture Identification Panel  

**Gram + Bacteria:**
- Enterococcus
- *L. monocytogenes*
- *Staphylococcus*
  - *S. aureus*
  - *Streptococcus*
    - *S. agalactiae*
    - *S. pyogenes*
    - *S. pneumoniae*

**Antibiotic Resistance:**
- *mecA*
- Van A/B
- KPC

**Gram - Bacteria:**
- *A. baumannii*
- *H. influenzae*
- *N. meningitidis*
- *P. aeruginosa*
- *Enterobacteriaceae*
  - *Enterobacter cloacae complex*
  - *E. coli*
  - *K. oxytoca*
  - *K. pneumonias*
- *Proteus*
- *S. marcescens*

**Yeast:**
- *C. albicans*
- *C. glabrata*
- *C. krusei*
- *C. parapsidosis*
- *C. tropicalis*

* FDA-Cleared for the first time
Matrix Assisted Laser Desorption Ionization – Time of Flight

- Identify bacteria/yeast/fungi/mycobacteria from colonies in minutes
  - BM got FDA approval for 173 organisms
- ID bacteria/yeast from positive blood culture bottles (not FDA approved)
- Replace gram stain of bacterial colonies
  - Not a reason to buy one but once you have one a potential good use
Impact of Rapid MALDI-TOF Results in Bacteremic Adults\textsuperscript{1}

- Intervention team: 2 ID physicians, 3 ID pharmacists, ID Pharmacy resident
- Team members received real time notification based on GS, ID, and susceptibility results from lab and communicated results to prescribers
  - MALDI results from colonies (not directly from BC broth)
- Made evidence-based antibiotic recommendations
- Compared 256 bacteremic results preintervention to 245 patients post intervention

\textsuperscript{1} Huang, AM et al. 2013. CID. 57: 1237-1245
Results of Intervention

<table>
<thead>
<tr>
<th></th>
<th>Preintervention</th>
<th>Postintervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to organism ID</td>
<td>84.0 hours</td>
<td>55.9 hours</td>
</tr>
<tr>
<td>Time to effective therapy</td>
<td>30.1 hours</td>
<td>20.4 hours</td>
</tr>
<tr>
<td>Time to optimal therapy</td>
<td>90.3 hours</td>
<td>47.3 hours</td>
</tr>
<tr>
<td>ICU stay</td>
<td>14.9 days</td>
<td>8.3 days</td>
</tr>
</tbody>
</table>
Rapid Bacterial Diagnosis
M. TB Complex
Rapid Molecular Detection of MTB Complex and Rifampin Resistance Directly in Respiratory Specimens

- MTB/RIF automated molecular (PCR) Test
  - Detects genes for MTB Complex (7 different Mycobacteria) and rifampin resistance (marker for multi-drug resistance)

Detected 551/561 culture positive, smear positive cases and 124/171 culture positive, smear negative cases

- Correctly identified 200/205 rifampin resistant bacteria and 504/514 rifampin sensitive bacteria

- May be used to replace AFB smears for respiratory specimens

Better Tests, Better Care: Improved Diagnostics for Infectious Diseases
Caliendo, A. M. et al. CID 2013. 57: S139-S170

In this IDSA policy paper, we review the current diagnostic landscape, including unmet needs and emerging technologies, and assess the challenges to the development and clinical integration of improved tests. To fulfill the promise of emerging diagnostics, IDSA presents recommendations that address a host of identified barriers. Achieving these goals will require the engagement and coordination of a number of stakeholders, including Congress, funding and regulatory bodies, public health agencies, the diagnostics industry, healthcare systems, professional societies, and individual clinicians.
Are We There Yet?

We are off the back roads and on the highway
Stay tuned for
“Fast Times at Technology High”