Rapid Bacterial Diagnostics – Are We There Yet?
Prof. Stephen Brecher, Boston University School of Medicine
A Webber Training Teleclass

Rapid Bacterial Diagnostics
Are We There Yet?

Stephen M. Brecher, Ph.D.
VA Boston Healthcare System
BU School of Medicine

Hosted by Nicole Kenny
Virox Technologies Inc.

Disclosures
• Bacterioscan – Advisory Board
• Cubist – Speakers Bureau, in-house training
• Merck – Speakers Bureau
• Theravance – Advisory Board
• 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
Which includes results communicated to the health care provider

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.

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?
What is “There”

• A single pathogen
• Multiple pathogens
• Any potential pathogen
• Complex disease pathogens (e.g. CF)
• Anatomical specific pathogens
• Antibiotic specific result: S or R

Desired Outcomes

• Reduce time to appropriate therapy
• Reduce length of stay
• Reduce transmission of pathogen
• Reduce cost

Goals

Micro/ID/Pharmacy/IC

Reduce time to appropriate treatment
Directed rather than empirical therapy

Improve patient care/reduce length of stay/decrease transmission via IC

Decrease emergence of antibiotic resistance/antibiotic stewardship

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

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“Working” Gram Stains
The Chodosh Method

- 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\(^1\)

- 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


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Predicting the Pathogen in Suctioned Respiratory Secretions

Mural Dyslexia
• “The inability to see the big picture”
• The value of an assay should be measured by its impact on patient outcome, not solely on its cost
  – Length of stay
  – Prescription drugs
  – Infection control
• The most expensive test is one that does not work

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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 x 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 as a Marker for 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 MR-Based Rapid Detection of Candidemia

Assay Design

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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

Detection of Pathogens from Positive Blood Culture Bottles

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

Understanding Positive Blood Cultures

- Often, very few organisms/ml of blood
- Bottles usually positive in 16-48 hours
- Gram stain + bottles, subculture and wait 24 hours to get colonies
- Set up ID and susceptibility
- Results available in another 24 hours

FilmArray

- Two minutes of hands-on time
- Results in about 1 hour
- From a positive blood culture bottle, 27 targets
- From a nasal swab, 20 viral and bacterial pathogens
- Closed System – Contamination is not an issue
- PCR based Molecular

Blood Culture Identification Panel FDA Cleared

- Gram + Bacteria:
  - Enterococcus
  - L. monocytogenes
  - Staphylococcus
  - S. aureus
  - Streptococcus
  - S. agalactiae
  - S. pyogenes
  - S. pneumoniae
- Antibiotic Resistance:
  - mecA
  - Van A/B
  - KPC
- Gram - Bacteria:
  - Enterobacteriaceae:
    - Enterobacter cloacae complex
    - E. coli
    - K. oxytoca
  - Proteus
  - S. marcescens
- Yeast:
  - C. albicans
  - C. glabrata
  - C. krusei
  - C. parapsilosis
  - C. tropicalis

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The Verigene BC-GP
Intended Use

- Verigene BC-GP is a multiplexed in vitro diagnostic test for the detection and identification of pathogenic gram-positive bacteria
- Verigene BC-GP is indicated for use in conjunction with other clinical and laboratory findings such as culture and is not used to monitor bloodstream infections
- Sub-culturing is necessary for susceptibility testing, identification of non-detected organisms, differentiation of mixed growth, association of resistance marker or epidemiological typing
- Our system detects and identifies the following bacterial genera/species and resistances

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus</td>
<td>faecium</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>faecalis</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>pyogenes</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>agalactiae</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>anginosus</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>pneumoniae</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>lugdunensis</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>epidermidis</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>aureus</td>
</tr>
</tbody>
</table>

Verigene BC-GP independently detects and identifies the following:

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas</td>
<td>aeruginosa</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>oxytoca</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>pneumoniae</td>
</tr>
<tr>
<td>Escherichia</td>
<td>coli</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>spp.</td>
</tr>
<tr>
<td>Citrobacter</td>
<td>spp.</td>
</tr>
<tr>
<td>Proteus</td>
<td>spp.</td>
</tr>
<tr>
<td>Serratia</td>
<td>marcescens</td>
</tr>
<tr>
<td>S. sonnei</td>
<td></td>
</tr>
<tr>
<td>S. dysenteriae</td>
<td></td>
</tr>
</tbody>
</table>

ICU stay
- Time to optimal therapy: 90.3 hours
- Time to effective therapy: 30.1 hours
- Time to organism ID: 84.0 hours
- Time to ICU stay: 14.9 days

Impact of Rapid MALDI-TOF Results in Bacteremic Adults

- 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

Results of Intervention

<table>
<thead>
<tr>
<th>Time to ID</th>
<th>Preintervention</th>
<th>Postintervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to effective therapy</td>
<td>36.1 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>

Matrix Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF)

- Identify bacteria/yeast/fungi/mycobacteria from colonies in minutes
- 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
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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

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 1
Parainfluenza 2
Parainfluenza 3
Parainfluenza 4
RBV

Bacterial
Bordetella pertussis
Chlamydia pneumoniae
Mycoplasma pneumoniae

Rapid Bacterial and Viral Diagnosis
Multi-Plex PCR

The Evidence

- Overall, 95% sensitivity and 99% specificity

<table>
<thead>
<tr>
<th>Clinical Sensitivity and Specificity of the FilmArray Respiratory Panel</th>
<th>Positive</th>
<th>Non-Positive</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A</td>
<td>95%</td>
<td>90%</td>
<td>95%</td>
</tr>
<tr>
<td>Influenza B</td>
<td>91%</td>
<td>95%</td>
<td>93%</td>
</tr>
<tr>
<td>Influenza A/H1</td>
<td>92%</td>
<td>93%</td>
<td>93%</td>
</tr>
<tr>
<td>Parainfluenza 1</td>
<td>95%</td>
<td>91%</td>
<td>94%</td>
</tr>
<tr>
<td>Parainfluenza 2</td>
<td>93%</td>
<td>92%</td>
<td>93%</td>
</tr>
<tr>
<td>Parainfluenza 3</td>
<td>93%</td>
<td>90%</td>
<td>92%</td>
</tr>
<tr>
<td>Parainfluenza 4</td>
<td>93%</td>
<td>92%</td>
<td>93%</td>
</tr>
</tbody>
</table>

Value and Use of Rapid Respiratory Multi-Plex Panels

- If assay is run on ER patients and the TAT is less than 2 hours, save money by answering the following questions
  - Viral? Bacterial?
  - Admit or not?
  - Antibiotics? Oseltamivir?
  - If admit, ? Precautions, IC protocols

“Lab’s Respiratory Panel Found to Curb Antibiotic Use”

The following quote is from CAP today with respect to the use of a respiratory panel PCR for pediatric patients in an ED

“Fewer children with respiratory disease symptoms hospitalized from the ED without a diagnosis, less antibiotic use, and a favorable ratio of reimbursement to expense…”

1. CAP Today January, 2014

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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

Directly from Stool
GI Pathogens

UTI Detection
Performance
- Fast Positive Detection (10 minutes)
  Sensitivity: 87.5%
  Specificity: 87.5%
  PPV: 51.9%
  NPV: 97.9%
- Elimination of Negatives (90 minutes)
  Sensitivity: 96.5%
  Specificity: 85.1%
  PPV: 49.2%
  NPV: 99.5%

Clinical Study conducted with St. Louis University Hospital. Study in July 2013 in 248 patients with 414 UTI positive at >1 x 10^4 CFU/mL, in processed and preserved-refrigerated specimens, showed 90% faster detection.

The Panels

GI Panel (In development: Not FDA Approved)

Bacteria:
- Diarrheagenic E. coli / Shigella
- Enteropathogenic E. coli (EPEC)
- Enterotoxigenic E. coli (ETEC)
- Enteroaggregative E. coli (EAEC)
- Enteroadherent E. coli (EAAE)
- Shiga-like toxin-producing E. coli (ETEC)
- Shigella Enteroinvasive E. coli (EIEC)

Protozoa:
- Cryptosporidium
- Cyclospora
- Entamoeba histolytica
- Giardia lamblia

Viruses:
- Adenovirus F 40/41
- Astrovirus
- Norovirus GI/GII
- Rotavirus A
- Sapovirus

GI Panel
(Meningitis Panel

Meningitis Panel

Lower Respiratory Panel

FilmArray Platform

After all panels are FDA-cleared, FilmArray will have assays covering 125 of the most common pathogens that cause death and disease.

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**Clostridium difficile**

- PCR has replaced EIA in CDI diagnostics
  - EIA was quick but not accurate

- About 10 FDA approved PCR assays
  - Rapid, accurate, very sensitive (rare false negatives), maybe too specific (false positives)
  - Test only patients at risk
    - *WBC, antibiotics, >3 BM/24h*
  - Test only loose stool (Brecher Guidelines)
  - “If it ain’t loose, it’s of no use”
  - “If the stick stands, the test is banned, if the stick falls, test them all”

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**Xpert® C. difficile PCR Test for Clostridium difficile**

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**Rapid Bacterial Diagnosis**

**M. TB Complex**

- 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


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**Rapid Molecular Detection of MTB Complex and Rifampin Resistance Directly in Respiratory Specimens**

- Compared isolation days and hospital costs in a retrospective analysis of a hypothetical cohort of 234 patients undergoing evaluation for possible TB
  - Compared results of 2 sputa AFB smears with 1 molecular PCR assay
  - PCR results available within hours; 2 smear results obtained at least 8 hours apart
  - PCR is significantly more expensive and more accurate than AFB smears
  - PCR reduced isolation bed utilization from 2.7 to 1.4 days per patient
  - System cost savings was over $500,000/year


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Can PCR Replace AFB Smears to Rule Out M. tuberculosis?

- Issues to consider
  - Cost
  - Prevalence
  - Severity of disease
  - How many specimens?
  - Quality of specimens

Whole Genome Sequencing

Clinical Insights from Metagenomic Analysis of Sputum Samples from Patients with Cystic Fibrosis

- Selected 3 patients and cultured/sequenced “climax” and “attack” communities over “clinical event” time sequences
  - Onset, treatment, post treatment, stable
  - Bacteria, viruses, fungi
  - Potential metabolic markers of different communities
- “…this pilot study provides an example of how metagenomic data might be used …. For the development of treatments tailored to individual patients”
  

Whole Genome Sequencing
Infection Control and Molecular Epidemiology

- WGS demonstrated that patient-to-patient transmission rarely accounts for acquisition of Staphylococcus aureus in an intensive care unit
- WGS used successfully to help unravel the CRE outbreak at NIH
- In the near future, WGS will be the method of choice for ME/IC
- ? Screen stool for fecal transplant

1. Price, JR. et al. 2014. CID. 58: 609-618

Volatile Organic Compounds

Diagnosis of Invasive Aspergillosis

- “In patients with suspected IFD, detection of a combination of farnesene, β-vatirenene, and cis-geranylacetone in the breath accurately and noninvasively discriminates IA patients from patients without IA.”
  - 54 immunosuppressed patients
  - Accurately dx 27/29 IA and 24/25 w/o IA
  - Sensitivity of 93%, Specificity of 96%


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Point of Care Testing

- R/O MTB in Pulmonary Clinics
- Group B Streptococci in OBGYN
- GC/Chlamydia, HIV, Herpes in STD Clinics
- Influenza and other respiratory viruses in the ER

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.

The Ultimate in Rapid The Selfie

Dr. Brecher,
I was a previously healthy 28 year old, very muscular male. I have traveled in Asia and Africa. I love the outdoors, have had numerous mosquito bites, and may have been exposed to Wuchereria bancrofti. I think I may have elephantiasis. Please see attached “selfie”. Thanks for your help.

Hans

Are We There Yet?

We are off the back roads
Stay tuned for
“Fast Times on the Technology Highway”

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