Multiplex Molecular Panels for Infectious Disease Diagnosis
Performance, Interpretation, and Cost-Effectiveness

Objectives

• Summarize the test characteristics of the available FDA-approved multiplex molecular panels that can simultaneously identify the most common pathogens implicated in respiratory viral, blood stream, GI, and CNS infections
• Highlight the advantages and limitations of multiplex technology for infectious diseases
• Discuss the potential utilization of these new comprehensive syndromic panels in clinical practice

General Comments

• Clinical syndromes are rarely specific for a single pathogen
• Diagnostic assays to rapidly and accurately identify the major pathogens responsible for clinical syndromes are a major unmet need
• In the absence rapid syndromic diagnostic tests, uncertainty about the etiology may prevent optimal patient management
• Multiplex syndromic panels reduce the need for multiple specimen collection, simply the testing algorithm, reduce time to result, improve sensitivity over conventional methods, and may detect pathogens that are not detected by conventional methods
  • Increased patient and physician satisfaction
General Comments

• For the lab, simplified workflow and consolidation of test methods can reduce costs
• Even when additional costs are incurred syndromic panels can improve testing efficiency, simplicity, and accuracy
• Increase cost can be mitigated by some manufacturers offering an option where clinicians can request a subset of tests on the panel
  • Pay only for tests performed
• Multiplex panels can improve public health by early recognition of outbreaks
  • EV D68, Cyclospora cayetanensis, Shigella sonnei

Multiplex Test Platforms

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Prodesse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>Real-time PCR</td>
</tr>
<tr>
<td>Degree of multiplexing</td>
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<tr>
<td>Panels</td>
<td>RP, GI</td>
</tr>
<tr>
<td>Complexity</td>
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</tr>
<tr>
<td>Automation</td>
<td>Partial</td>
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<tr>
<td>Throughput</td>
<td>Medium</td>
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<td>~3-4h</td>
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### BD Max

<table>
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<td>Panels</td>
<td>GI, Vaginitis</td>
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<tr>
<td>Complexity</td>
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<tr>
<td>Automation</td>
<td>Full</td>
</tr>
<tr>
<td>Throughput</td>
<td>Low-moderate</td>
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### Luminex

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<tr>
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<tbody>
<tr>
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<td>PCR with liquid phase bead array</td>
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<td>Degree of multiplexing</td>
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<tr>
<td>Panels</td>
<td>RP, GI</td>
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<tr>
<td>Complexity</td>
<td>High</td>
</tr>
<tr>
<td>Automation</td>
<td>Partial</td>
</tr>
<tr>
<td>Throughput</td>
<td>Moderate-high</td>
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### BioFire FilmArray

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<td>Nested PCR with melt curve analysis</td>
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<td>Degree of multiplexing</td>
<td>14-22 targets</td>
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<tr>
<td>Panels</td>
<td>RP, GI, BCID, CNS, LRTI*</td>
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<tr>
<td>Complexity</td>
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<tr>
<td>Throughput</td>
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<td>Analysis time</td>
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*FDA clinical trial in progress
### Verigene

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<td>Automation</td>
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<tr>
<td>Throughput</td>
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### GenMark eSensor/ePlex

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<td>RP, BCID*</td>
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<td>Analysis time</td>
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*FDA clinical trial in progress

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**Respiratory Panels**
Upper Respiratory Tract Infections

- One of the leading causes of morbidity and mortality worldwide
- Cause a range of symptoms, from mild to life-threatening
- Extremely common
  - <5 years old: mean 6.1 episodes/year
  - >40 years old: mean 4.1 episodes/year
- Caused by a variety of pathogens often with overlapping clinical syndromes including:
  - Viruses
    - INF A/B, RSV, PIV, ADV, HRV, COV, MPV,
  - Bacteria
    - *Bordetella pertussis* and *parapertussis*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*

Respiratory Virus Detection Past State

Historically, performed using a variety of methods:
- Culture
- Antigen testing
- FA
- LFIA
- Individual or limited multiplexed NAATs

Respiratory Panel Advantages

- Simple sample-to-answer methods available with minimal hands-on time
- Consolidation of diagnostic methods with reduction in laboratory costs
- Rapid analysis time (as short as <60 minutes)
- Broad coverage
- Greater diagnostic yield
- Local epidemiology
- Enhanced infection control measures

Respiratory Panel Advantages

- Comprehensive diagnostic test can lead to improved use of antibiotics/antivirals and reduced use of ancillary diagnostic tests and LOS
- Allow for detection/identification of viruses (e.g., hMPV, coronaviruses) not routinely detected by conventional methods
- Multiplex respiratory panels should be particularly useful in high-risk patient populations
  - Infants and young children
  - Elderly
  - ICU patients
  - Immunosuppressed
  - Chronic lung disease

Respiratory Pathogen Detection Counts

<table>
<thead>
<tr>
<th>Date</th>
<th>Adenovirus</th>
<th>Bacteria</th>
<th>Enterovirus</th>
<th>Parainfluenza</th>
<th>Rhinovirus</th>
<th>RSV</th>
<th>Staphylococcus</th>
<th>Streptococcus</th>
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</table>
Respiratory Panel Limitations

- High cost to patient and institution
  - Consider rapid tests for Inf A/B and RSV as first line tests
- Robust clinical and economic impact studies are lacking
- Most panels do not allow customized ordering
  - Verigene and Luminex are exceptions
- While comprehensive, do not include all possible pathogens
- Co-detections can be difficult to interpret
- Suboptimal performance for some assays included in panel
  - Adenoviruses, Influenza viruses
- Potential false positives due to amplicon or target carryover
- Reimbursement?
  - Medicare CPT 87633 $514.55
  - Palmetto GBA draft Limited Coverage Determination

PROPOSED/DRAFT Local Coverage Determination (LCD): MolDX: Multiplex Nucleic Acid Amplified Tests for Respiratory Viral Panels (DL37713)

The use of highly multiplexed NAAT tests as front-line diagnostics cannot be justified at the current time.

- A panel that includes pathogens that are very rare, or a panel in which all pathogens do not cause overlapping clinical syndromes, or when some pathogens are found only in specific patient populations (immunocompromised patients) is not reasonable and necessary.
- Despite an individual patient having signs or symptoms of a respiratory illness, the above highly multiplexed NAAT tests are not reasonable and necessary: a one size fits all diagnostic approach.
- The use of limited simplex or multiplex direct probe technique tests for respiratory viruses, such as Influenza A/B with, or without inclusion of RSV, is a Medicare covered benefit.
- Comment period 3/26/18-5/10/18.
MUSC Current State

POCT
- INF A/B Ag (Digital IA)
  - 4000/year; $12.30/test; MC, $16.33; Charge, $200
- RSV Ag (Traditional IA)
  - 240/year; $10.50/test; MC, $16.33; Charge, $266

Laboratory
- FilmArray RP
  - INF A, INF B, ADV, COVs, PIVs, RSV, MPV, HRV/HEV (Bp, Cp, Mp)
  - 7,000/year; $119/test; MC, $514.55; Charge, $865
- Approximately $900,000 annual cost

FilmArray RP Utilization Review

Utilization by Location 6/1/16-6/1/17
- 33 locations ordered ≥50 RPs (83%)
- 113 locations ordered <50 RPs (27%)

MUSC Utilization Review

Engage stakeholders in dialogue about medical necessity of RV diagnostics in various patient populations
- ED, Outpatient clinics, Hospitalized patients
- Need for limited panel (INF A/B and or RSV)

Review POCT needs (Ag detection vs. NAATs)
- Standardized ag detection tests (INF A/B, RSV, GAS)

Develop MUSC best practices for use of RV diagnostics.
- Restrict use to immunocompromised, patients acutely ill who are potential hospital admissions or at risk for complications, and critically ill patients in hospital, particularly in the ICUs?
- Need for targeted panel (INF A/B and/or RSV)?
- Need for stand alone Bordetella pertussis assay?
Blood Culture Identification Panels

Time Lines for ID and AST of Blood Culture Isolates

- Standard Testing: 12–72 h
- Pathogen ID: 5 min
- Antimicrobial Susceptibility Testing: 24–72 h

Definitive identification of a pathogen can take 24 to 72 hours through traditional culture methods. This delay can lead to inappropriate or overly broad antimicrobial therapy and result in therapy-related complications, antimicrobial resistance, and increased patient morbidity, mortality, and costs.

FDA-cleared BCID Panels

- **FilmArray**
  - 1 panel, 23 pathogens and 4 AMR genes
  - PCR amplification with melt curve analysis

- **Verigene**
  - GP panel, 12 pathogens and 3 AMR genes
  - GN panel, 8 pathogens and 6 AMR genes
  - Solid-phase array (no amplification)

- **GenMark**
  - GP, GN and FP panels currently in FDA clinical trials
  - Most comprehensive panels
  - PCR amplification with electrochemical detection

Study Design and Patient Population

Inclusion Criteria
› Hospitalized patients (≥ 18 years of age) with positive blood culture(s)
› August 1, 2010 to October 31, 2014

Exclusion Criteria
› Contaminated blood cultures
› Organisms not included on the BCID panel
› Patients who expired (or placed on hospice care) prior to blood culture positivity
› Patients transferred from an outside facility with previous blood cx of the same organism

Control Group
(8/1/10 to 10/31/10)
No formal review of positive blood cultures by ASP

Stewardship
(8/1/12 to 10/31/12)
Prospective review of blood cultures by ASP (no BCID)

Stewardship + BCID
(8/1/14 to 10/31/14)
BCID with real-time alert coupled with ASP intervention

Timeline for Microbiology and Antimicrobial Therapy Among Study Groups

Study Findings and Conclusions

• In patients with bloodstream infections, real time stewardship intervention resulted in higher rates of antibiotic de-escalation
• The addition of rapid organism identification to stewardship intervention significantly shortened the time to effective therapy and further improved antibiotic streamlining compared to stewardship intervention alone
• Groups did not different in LOS, mortality, readmission, or costs
• At institutions with low rates of antibiotic resistance, the greatest benefit of rapid microbiologic tests may be in limiting unnecessary antibiotic use
**BCID Limitations**

Separate panels for GP, GN, and FP depend upon results of initial Gram stain
- May miss mixed infections
- Not all organisms causing sepsis and associated AMR genes are represented on panels
- Supplements but does not replace conventional identification and AST methods
- Mixed cultures of *Staphylococcus aureus* and CNS may lead to ambiguous results for presence of MRSA
  - *mecA* gene is not assigned to species
  - Poor differentiation between *Streptococcus pneumoniae* and viridans streptococci
- False positive results due to contamination of blood culture medium with bacterial DNA
  - *e.g.* Pseudomonas, Proteus

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**Gastrointestinal Panels**

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**Gastrointestinal Infections**

- Gastroenteritis is one of the most common infectious disease syndromes accounting for 175 million cases and 25 million outpatient visits in the US annually
- There are a number of bacteria, viruses, and parasites that can cause gastrointestinal infections with overlapping clinical presentations
- Prevalence varies with the patient’s age, immune status, and travel/exposure history
- Most cases are self-limited and do not require treatment or testing
- However, some pathogens can cause severe, life-threatening infections, especially in immunocompromised patients
- Definitive ID is necessary to guide therapy and infection prevention and control measures
Detection of GI Pathogens

Traditional laboratory testing for these pathogens consists of exams performed on stool specimens, commonly including:

- Bacterial stool culture
- Ova and parasite exam
- Antigen detection methods for Cryptosporidium, Giardia, and rotavirus
- PCR tests for norovirus, adenovirus 40/41

These tests range in price and can take several days until a result is produced.

Multiplex Molecular GI Panels

- Allows for simplified ordering and testing
- Now several FDA-approved/cleared options
- Detect common bacterial, viral, and protozoal pathogens
- Test results available within hours
- Benefits of these panels are recognized by the ACG*
  
  “Molecular diagnostic tests can provide a more comprehensive assessment of disease etiology by increasing the diagnostic yield compared with conventional diagnostic tests.”

*Riddle MS, DuPont HL, Connor BA. 2016 Am J Gastroenterol;111:602–622

GI Panel Advantages

- Rapid
- Similar or increased sensitivity compared to conventional methods
- Detect viruses and bacteria for which testing is not routinely available
  - EPEC, ETEC, EAEC
  - Sapovirus, Astrovirus
- Detect more potential pathogens than conventional methods

GI Panel Limitations

- Expensive
  - A patient may be charged >$1000 for a single test
- Results may be difficult to interpret
  - Detection of multiple potential pathogens
  - Colonization, carriage, or infection?
  - Inclusion of C. difficile in some panels is controversial
  - Clinical correlation is required
- Bacterial isolates not available for public health testing (e.g., typing assays)
  - Residual specimen should be saved when possible for additional testing
- Reimbursement
  - Medicare CPT 87507, $514.55 (draft LCD to deny coverage)
  - Private payers may also deny coverage
Laboratory Testing for Infectious Causes of Diarrhea

Central Nervous System Panels

Meningitis and Encephalitis

- Potentially devastating conditions caused by bacteria, viruses and fungi
- Clinical presentation usually non-specific
  - Fever, headache, altered mental status, +/- nuchal rigidity
  - CSF WBC with differential, protein level, and glucose concentration (bacterial vs. viral infection)
  - Microbiologic testing is required for a definitive diagnosis
- Laboratory diagnosis
  - CSF Gram stain and culture (Bacteria)
  - Targeted NAATs (HSV, Enteroviruses)
  - Antigen and antibody detection (Cryptococcus, Arboviruses)
Meningitis/Encephalitis Panel

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FilmArray Meningitis/Encephalitis panel</th>
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<tbody>
<tr>
<td>Pathogens detected</td>
<td>Cytomegalovirus, enterovirus, herpes simplex virus 1, herpes simplex virus 2, human herpesvirus 6, human parvovirus, varicella-zoster virus, echovirus (all 9), herpes viruses infecting, enterovirus meningovascular, Neisseria meningitidis, Streptococcus agalactiae, Streptococcus pneumoniae</td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
</tr>
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</table>
| Fungi | Cryptococcus neoforans-C.

M/E Panel Advantages

- Broad panel
  - Inclusion of agent for which routine testing is not available (e.g., HPeV)
- Rapid TAT compared to standard methods (e.g., culture)
- Potentially more sensitive in patients who received antibiotics prior to CSF collection
- Rapid assay with minimal technologist hands-on time

M/E Panel Limitations

- Cost
  - Targeted testing may be more appropriate in certain cases
- Broad, but not all inclusive!
  - Staphylococcus spp., Cutibacterium acnes, other Gram-negative bacteria
- Interpretation of positive results may be challenging
  - HHV6 and CMV – Latent infection vs. true cause of CNS disease
  - S. pneumoniae – False-positive results have been reported
  - ≥2 targets detected
- CSF Gram stain and culture are still required
  - Susceptibility testing, public health laboratory requirements
Curetis Unyvero System LRTI Cartridge

First FDA-cleared syndromic panel for this indication (4/4/18)
Sample to answer system with results in 5 hours
Detects 30 bacterial pathogens and 10 antimicrobial resistance genes

FilmArray LRT Infection Panel*

*LDA clearance pending
Summary

• Syndromic panels are novel, powerful tools that assist in timely diagnosis and influence individual patient management, antimicrobial stewardship and infection control and prevention
• It is anticipated that syndromic testing will be used more in the future
• Concerns about "one-size fits all" approach are misplaced considering that clinicians rarely ordered cultures for specific organisms and is not in line with clinical practice

• In scenarios where multiple individual tests would be ordered multiplex panels may actually be more cost effective
• Offering individual molecular assays for a few key pathogens such as C. difficile, Inf A/B and RSV may help with overuse of syndromic panels
• Establishment of clear algorithms and guidelines for ordering and interpreting these panels is necessary to inform their effective use
• Reimbursement by Medicare and private payers is being addressed by professional organizations
  • ASM, AMP, APHL, CAP, IDSA, PASCV