Meningitis and Encephalitis
Molecular Diagnostic Panels

How do they perform and When should they be used?

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Disclosures

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5R01AI117035-02
Central Nervous System (CNS) Infection

**Meningitis**
- Meningeal inflammation
  - ↑ WBCs in CSF
  - Plus some combination of:
    - Fever
    - Headache
    - Stiff neck
    - Altered mental status

**Encephalitis**
- Parenchymal Inflammation
  - Altered mental status
  - Plus at least 2 of:
    - Fever
    - Seizures
    - CSF WBC ≥ 5 cells/mm³
    - Abnormal imaging or EEG

**Meningoencephalitis (ME)**
Epidemiology

• **Multiple etiologies**
  - Infectious and non-infectious causes
  - Signs/symptoms non-specific

• **Uncommon**
  - Meningitis: 4-30 cases /100,000 annually
  - Encephalitis: 3-7 cases /100,000 annually

• **Vaccine and public health interventions**
  - Viral meningitis - mumps previously most common, CNS complications of measles and VZV have declined
  - Bacterial meningitis – reduction in cases from 10,000-20,000/year to < 4200 annually

Thigpen et al. NEJM 2011; 364: 2016
Traditional Diagnostics

• CSF studies
  - Cell count, glucose, and protein
  - Gram stain
    ✓ $10^4$ CFU/ml limit of detection; 10-93% sensitive
  - Culture
    ✓ $10^2 – 10^3$ CFU/mL limit of detection; 60-90% sensitive
  - NAAT for viruses (only HSV and Enterovirus FDA cleared)
  - Antigens and serologies

• Other tests
  - Blood culture
  - Biomarkers – procalcitonin and CSF lactate
Syndromic Molecular Panels

• Capitalize on the power of multiplexed NAAT

• Commercial tests (US market)
  - FDA cleared
    ✓ Biofire FilmArray: 14 pathogens (6 bacteria, 7 viruses, 1 yeast)
      200 µL CSF run over 1 hour; cost ≅ $200
  - In development
    ✓ GenMark ePlex: bacterial, viral, fungal targets
      Sample to result 60-90 min; cost ≅ ???

• Laboratory developed tests (LDTs)
# The FilmArray (FA) Panel

## Bacteria
- *Escherichia coli K1*
- *Haemophilus influenzae*
- *Listeria monocytogenes*
- *Neisseria meningitidis*
- *Streptococcus agalactiae (GBS)*
- *Streptococcus pneumoniae*

## Viruses
- *Cytomegalovirus (CMV)*
- *Enteroviruses (EV)*
- *Human herpes virus 6 (HHV-6)*
- *Human parechovirus (PeV)*
- *Herpes simplex virus 1 (HSV-1)*
- *Herpes simplex virus 2 (HSV-2)*
- *Varicella zoster virus (VZV)*

## Yeast
- *Cryptococcus neoformans*
- *Cryptococcus gattii*
## The FilmArray (FA) Panel

### Limit of Detection

<table>
<thead>
<tr>
<th><strong>Bacteria</strong></th>
<th><strong>Limit of Detection</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli K1</em></td>
<td>$10^3$ CFU/mL</td>
</tr>
<tr>
<td><em>Neisseria meningitidis</em></td>
<td>$10^2$ CFU/mL</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>$10^3$ CFU/mL</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae (GBS)</em></td>
<td>$10^3$ CFU/mL</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>$10^3$ CFU/mL</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>$10^2$ CFU/mL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Viruses</strong></th>
<th><strong>Limit of Detection</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytomegalovirus (CMV)</td>
<td>100 TICD$_{50}$/mL, 4.3 x 10$^3$ copies/mL</td>
</tr>
<tr>
<td>Enteroviruses (EV)</td>
<td>5-50 TICD$_{50}$/mL</td>
</tr>
<tr>
<td>Herpes simplex virus 1 (HSV-1)</td>
<td>250 TICD$_{50}$/mL, 1.5 x 10$^3$ copies/mL</td>
</tr>
<tr>
<td>Herpes simplex virus 2 (HSV-2)</td>
<td>50 TICD$_{50}$/mL, 1.3 x 10$^3$ copies/mL</td>
</tr>
<tr>
<td>Varicella zoster virus (VZV)</td>
<td>LoD 1.25 – 40 TCID$_{50}$/mL</td>
</tr>
<tr>
<td>Human herpes virus 6 (HHV-6)</td>
<td>4.3 x 10$^3$ copies/mL</td>
</tr>
<tr>
<td>Enteroviruses</td>
<td>5-50 TICD$_{50}$/mL</td>
</tr>
<tr>
<td>Human parechovirus (PeV)</td>
<td>LoD 1 – 40 TCID$_{50}$/mL</td>
</tr>
<tr>
<td>Herpes simplex virus 1 (HSV-1)</td>
<td>0.1 TICD$_{50}$/mL, 1.7 x 10$^3$ copies/mL</td>
</tr>
<tr>
<td>Cytomegalovirus (CMV)</td>
<td>Less sensitive than LDT</td>
</tr>
<tr>
<td>Enteroviruses (EV)</td>
<td>Less sensitive than CrAG</td>
</tr>
<tr>
<td>Herpes simplex virus 1 (HSV-1)</td>
<td>0.1 TICD$_{50}$/mL, 1.7 x 10$^3$ copies/mL</td>
</tr>
<tr>
<td>Varicella zoster virus (VZV)</td>
<td>1.7 x 10$^3$ copies/mL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Yeast</strong></th>
<th><strong>Limit of Detection</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>10$^2$ CFU/mL</td>
</tr>
</tbody>
</table>

FA, Simplexa and Xpert EV package inserts; Hanson et al. JCM 2016; 54(3): 785
Multicenter Clinical Study

- 11 sites in the US
- 1560 residual CSF specimens
- Calculations of test performance
  - Bacteria vs. conventional culture
  - Viruses and yeast vs. PCR with sequencing
  - Discordant results
    - Repeat molecular testing when possible
    - Blinded review of demographic, clinical, and laboratory data

Leber et al. JCM 2016; 54(9): 2251
Results

• Low overall positivity rate (7.6%; 119/1560)
  - 10 of 14 targets < 10 cases
  - No confirmed *Listeria* or *Neisseria*

• Total agreement
  - 84.4% positive and > 99.9% negative

• False negatives
  - 1 GBS, 2 EV, 3 HHV-6

• Additional confirmed detections n=21
## False Positive Results (n=22)

<table>
<thead>
<tr>
<th>Target</th>
<th>Confirmed positives/Total positives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>2 of 3 (66%)</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>2 of 2 (100%)</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>No detections</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>No detections</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td>0/1</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>9 of 16 (56%)</td>
</tr>
<tr>
<td>CMV</td>
<td>4 of 6 (66%)</td>
</tr>
<tr>
<td>EV</td>
<td>49 of 51 (96%)</td>
</tr>
<tr>
<td>HSV-1</td>
<td>2 of 4 (50%)</td>
</tr>
<tr>
<td>HSV-2</td>
<td>11 of 12 (92%)</td>
</tr>
<tr>
<td>HHV-6</td>
<td>19 of 22 (79%)</td>
</tr>
<tr>
<td>HPeV</td>
<td>12 of 12 (100%)</td>
</tr>
<tr>
<td>VZV</td>
<td>6 of 7 (86%)</td>
</tr>
<tr>
<td><em>Cryptococcus</em> spp.</td>
<td>3 of 5 (60%)</td>
</tr>
</tbody>
</table>
Potential Impact
Patient Care

Case 1
• Initial presentation
  - 75 year old Vietnamese man
  - Confusion + speech difficulties x 2 wks
  - Lymphoma, chemo 3 months ago
  - No focal deficit and MRI normal
  - CSF 210 WBCs (72% PMNs), FA HSV-1 +
  - Rx acyclovir but progressive neurologic decline
• Transfer to another hospital
  - LDT HSV PCR –
  - MRI basilar leptomeningeal enhancement
  - TB PCR and cultures positive (day 7 and 13, respectively)
  - Original CSF obtained: FA -, HSV PCR -,
    TB PCR +

Case 2
• Initial presentation
  - 63 years kidney transplant (2012)
  - Headache and neck pain x 3 wks
  - Progressively altered mental status despite normal MRI and antimicrobials
  - No recent travel or exposures, September
• Transfer to another hospital
  - Febrile 40°C with rigors
  - Confused with no focal deficit
  - CSF WBC 662 (84% PMNs)
  - FA Cryptococcus +
• Result several hours before CrAg and days before culture

More rapid time to identification
• High clinical suspicion for bacterial meningitis
• Immunocompromised hosts

Caution
• Contamination and untargeted agents of disease
• Latent viruses

Gomez et al. OFID DOI: 10.1093/ofid/ofw245
Potential Impact
Public Health

- Culture independent diagnostic tests (CIDTs)
  - May improve disease estimates
    ✓ Potentially more sensitive than culture
    ✓ More patients tested
    ✓ Detect polymicrobial infections
  - Caution with estimates
    ✓ Changes in testing practices
    ✓ Differences in test characteristics
    ✓ Case definitions do not include clinical symptoms and rely on culture
  - Emerging Infections Program surveys
    ✓ < 1% of ABCs offer these tests for ME pathogens (2014-2015)

Langley et al. EID 2015; 21(9)
Conclusions & Recommendations

Clinical Laboratories
1. Targeted testing
2. Contamination control
3. Monitor positivity rates and potentially confirm

Public Health
1. Monitor CIDT usage trends
2. Revaluate case definitions for reportable diseases
3. Help monitor performance characteristics