Meningitis/Encephalitis Panel

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Disclosure

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April 30, 2019

No relevant financial relationships do disclose.
Objectives

• Definitions
• Types of meningitis
• Causes of meningitis
• Review of the BioFire M/E Panel
• Case studies associated with the M/E Panel
• Challenges associated with the M/E Panel
Participant Question

How many of you use a M/E panel in your laboratory or facility?

A. We use the M/E panel.

B. We do not use the M/E panel.
Definitions

Meningitis is an inflammation (swelling) of the protective membranes (meninges) covering the brain and spinal cord.

Encephalitis is an inflammation of the brain.
Meningitis can kill or disable in a matter of hours.
MENINGITIS
FACTS & FIGURES
#AfterMeningitis #WorldMeningitisDay

THE GLOBAL BURDEN
Meningitis affects over 2.8 million people a year from all over the world.

ANYONE OF ANY AGE
Anyone of any age can be affected by meningitis. Infants, young children, teens and older people are at greater risk.

ACT FAST
Meningitis can kill or disable in 24 hours. Always trust your instincts.

AFTER EFFECTS
Meningitis and septicaemia after effects can include deafness, organ failure, limb loss, cerebral palsy, brain damage and epilepsy.
Type of Meningitis

1. Viral Meningitis
2. Bacterial Meningitis
3. Fungal Meningitis
4. Parasitic Meningitis
5. Amebic Meningitis
6. Non-Infectious Meningitis
Meningitis/Encephalitis in US, 2011-2014

Percentage

- Enterovirus 50.9%
- Unknown 18.7%
- Bacteria 14.1%
- Herpes 8.3%
- Noninfectious 3.5%
- Arboviruses 1.1%
- Other viruses 0.8%
- Fungal 2.7%

N = 26,429

Causes of Viral Meningitis

1. **Enteroviruses** – non-polio enteroviruses, coxsackieviruses, echoviruses
2. **Herpesviruses** – HSV-1, HSV-2, HHV-6, VZV, CMV, EBV
3. **Arboviruses** (West Nile virus)
4. **Lymphocytic Choriomeningitis Virus**
Causes of Bacterial Meningitis

- *Neisseria meningitidis*
- *Streptococcus pneumoniae*
- *Haemophilus influenzae*
- Group B Streptococcus
- *E. coli*
- *Listeria monocytogenes*

On average, bacterial meningitis caused about 4,100 cases and 500 deaths in the United States each year between 2003 and 2007.

Causes of Fungal Meningitis

- *Cryptococcus neoformans/gattii*
- *Histoplasma capsulatum var. capsulatum*
- *Blastomyces dermatitidis (B. gilchristii)*
- *Coccidioides immitis/posadasii*
Laboratory Detection of Infectious Agents Causing Meningitis

- Culture
- Serology
- Microscopy
- Antigen Detection
- Nucleic Acid Amplification Tests
Bacterial Meningitis

<table>
<thead>
<tr>
<th>Table 8 Comparison of Results of RT-PCR and cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cultures (N = 56)</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>RT-PCR: +</td>
</tr>
<tr>
<td>2 (3.5%)</td>
</tr>
<tr>
<td>23 (41.1%)</td>
</tr>
<tr>
<td>25 (44.6%)</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>3 (5.4%)</td>
</tr>
<tr>
<td>28 (50.0%)</td>
</tr>
<tr>
<td>31 (55.4%)</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>5 (8.9%)</td>
</tr>
<tr>
<td>51 (91.1%)</td>
</tr>
<tr>
<td>56</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 13.885, \ P < 0.05. \]

*E. coli, S. aureus, L. monocytogenes, N. meningitidis, H. influenzae, S. pneumoniae, S. agalactiae*

Wang et al. *BMC Pediatrics* 2014, 14:224
BioFire M/E Panel Targets

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

**Viruses**
- Cytomegalovirus (CMV)
- Enterovirus
- Herpes simplex virus 1 (HSV-1)
- Herpes simples virus 2 (HSV-2)
- Human herpesvirus 6 (HHV-6)
- Human parechovirus
- Varicella zoster virus (VZV)

**Fungi**
- *Cryptococcus neoformans/gattii*
The FilmArray Pouch

- Reagent Reservoirs in Fitment
- 120 x 1μL Assay Wells
- bead beating
- magnetic beads
- amplicon dilution
- nested amplification & melt curve analysis
- multiplex PCR
Early PCR in a Pouch - 1993
Nested Multiplex PCR

- **Sample Injection Port**
- **DNA/RNA Purification**
- **Cell Lysis**
- **PCR I**
- **PCR II**
- **Water Injection Port**
- **Dilute 100x**

The diagram illustrates the process of Nested Multiplex PCR, showing the steps from cell lysis to the final PCR II stage with dilution.
Nested Multiplex PCR

Nested PCR

First outer primer set

Targeted sequence

1st Amplicon

Second inner primer set

Enter 2nd PCR

2nd Amplicon

PCR I

PCR II
Automated Results Analysis

- 102 Individual 2\textsuperscript{nd} Stage PCR Wells
- Each Well Contains One Reaction
Automated Results Analysis

- All Targets Tested in Triplicate
Automated Results Analysis

B. Normalized Melting Curves

- Double-stranded DNA (ds DNA)
- Single-stranded DNA (random coils)

Fluorescence (Normalized)

Temperature [°C]

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>76</td>
<td>100</td>
</tr>
<tr>
<td>79</td>
<td>75</td>
</tr>
<tr>
<td>82</td>
<td>50</td>
</tr>
<tr>
<td>88</td>
<td>25</td>
</tr>
<tr>
<td>91</td>
<td>0</td>
</tr>
</tbody>
</table>

Double-stranded DNA:
- gaattc
- cttaag

Single-stranded DNA:
- gacttc
- ctgaag

TM
S. pneumoniae was detected in 5/12 FP specimens using an independent PCR assay; additional information regarding seven unconfirmed FP specimens is detailed below in Table 10.

S. pneumoniae was detected in 5/12 FP specimens using an independent PCR assay; additional information regarding seven unconfirmed FP specimens is detailed below in Table 10.
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Count</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterovirus (CMV)</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>HSV-2 (HSV-1)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>VZV</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>HHV-6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Human Parechovirus</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>4</td>
<td><em>(E. coli K1)</em></td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td>2</td>
<td><em>(H. influenzae)</em></td>
</tr>
<tr>
<td><em>L. Monocytogenes</em></td>
<td>1</td>
<td><em>(N. meningitidis)</em></td>
</tr>
<tr>
<td><em>C. neoformans/gattii</em></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

September 2017 – March 2019 – 222 CSF specimens tested

( ) = have not detected
Meningitis/Encephalitis in US, 2011-2014

Percentage

- Enterovirus 50.9%
- Herpes 8.3%
- Arboviruses 1.1%
- Unknown 18.7%
- Bacteria 14.1%
- Noninfectious 3.5%
- Other viruses 0.8%
- Fungal 2.7%

N = 26,429
29 y/o Male Presents to ED

- Severe headache last 48 hours, no effect with ibuprofen, Tylenol
- Hurts to move eyes, positive Brudzinski’s (knees & hips flex when neck is flexed)
- Fever last 48 hours, ibuprofen and Tylenol did bring fever down
- Sore throat, no rash
- Labs: PCT, CBC, CMP, glucose all normal; bacterial culture and M/E panel performed
- CT of head is negative

- LP CSF: clear/colorless, nucleated cells 16 cells/µL, 70% lymph. 12% PMNs, glucose 60 mg/dL, protein 70.3 mg/dL

- ER doc discussed with ID, likely viral, but start ceftriaxone, vancomycin, and acyclovir and observe until initial microbiology results are available.

  Short time later

- CSF cytospin Gram stain few PMNs and NOS
- M/E panel positive for Enterovirus only
- ER doc discussed with ID; only conservative management needed, stop antibiotics and acyclovir, discharged patient (virology etiology) with prescription meds to control headache and instructions to return to ED if symptoms worsen.
54 y/o Male Presents to ED Twice

- 54 y/o male, ED with AMS, left sided facial pan and paresthesia (tingling)
- Admitted, extensive testing, nothing found, discharged home
- Children say he is still “out of it”
- Next day back to a different ED for evaluation
- LP – CSF is clear, colorless, 2 nucleated cells/μL, 2 RBCs/μL, glucose 85 mg/dL (serum 144 mg/dL), protein 102 mg/dL.

- CSF cytospin Gram stain no PMNs and NOS
- M/E panel positive for HHV-6 only
- MRI brain show numerous foci in periventricular and subcortical white matter, not specific for any particular condition
- Admitted because of facial and upper extremity paresthesia, confusion and a diagnosis of viral meningitis
- Condition resolved and discharged after 48 hours with neurology follow up

CSF quant. HHV-6B, 14,900 copy/mL
Serum quant. HHV-6B, > 999,000 copy/mL

HHV-6B causes roseola, >90% pop. infected early in life. Reactivation can cause encephalitis. Latent or integrated virus can confound interpretation of DNA detection.
Our Next M/E Panel Test – 5 days Later

- 13 y/o M, outside ED with severe headache of several days
- Fever
- LP – CSF is clear, colorless, 1 nucleated cells/µL, 412 RBCs/µL, glucose 61 mg/dL (serum 95 mg/dL), protein 38 mg/dL.
- CSF Gram stain NOS

- CSF referred us for M/E panel testing – positive for HHV-6 only
- **Repeat M/E panel on different tube of CSF – positive for HHV-6 only**
- No HHV-6 viral loads or typing performed
M/E Panel Laboratory Precautions

Process all specimens in a biological safety cabinet.
To guard against contamination of the specimen or test pouch due to nucleic acid/organism from the operator, other specimens, or cultured organisms in the laboratory.

Dedicated use of biological safety cabinet for sample preparation and pouch loading.
Cabinet should not be used for culture setup, examination of growing cultures, and is ideally used for molecular testing only.

Process one specimen and pouch at a time.
To prevent sample to sample contamination.

Wear a new disposable laboratory coat when performing M/E panel testing.
To guard against contamination of the M/E panel testing environment due to target material on the operator’s coat.
M/E Panel Specimen
Collection and Handling Precautions

Wear sterile gloves and a procedure mask when collecting CSF.
To guard against contamination of the CSF tubes with target material.

Wear clean gloves and procedure mask when handling CSF.
To guard against contamination of the CSF tubes with target material.

Avoid using CSF tubes that have been handled and used for testing in other parts of the laboratory.
To guard against contamination of the CSF tubes with target material.
M/E Panel Post Test Review

Correlate the results of CSF testing on the M/E panel with other laboratory data, clinical presentation and patient history.

All unexpected or atypical results should be examined carefully and repeat M/E panel testing may be warranted.
Participant Question

For those that use the M/E panel, what were the reasons for bringing the test into the laboratory?

A. Laboratory leaders decided that it would be a very useful test in our facility.

B. Physicians requested the test and wanted the test to be performed on site.
Participant Question

For those that use the M/E panel, what feedback have you received from clinicians about the test?

A. They like the test and are happy with the test results they receive.

B. They do not like the test and say that they never order the test when they have suspected meningitis cases.

C. They only use the test on patients they suspect of having viral meningitis because they believe that if it is bacterial meningitis the organism will grow and be seen on CSF cytospin Gram stain.
Question/Discussion Point

For those who use the BioFire M/E panel, do you use a special workflow in your laboratory for this test?

A. We use a special workflow when we perform M/E panel testing.

B. We do nothing different from other molecular tests when we run the M/E panel.

Is a special workflow needed when performing the BioFire M/E Panel?
Question/Discussion Point

For those who use the BioFire M/E panel, do you use some type of post-test review of patient clinical and laboratory data prior to reporting the test result?

A. We utilize a post-test review algorithm prior to reporting M/E panel test results.

B. We just report the M/E panel result once the test is finished.

Is a post-test result review needed when performing the BioFire M/E Panel?
### Question/Discussion Point

Do you think the organisms targeted by the BioFire M/E panel are appropriate?

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<th>Viruses</th>
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</table>

A. Yes  
B. No

Are the organisms targeted by the BioFire M/E panel appropriate?
Question/Discussion Point

For those who use the BioFire M/E panel, do you enforce the package insert comment which states that the panel is “...intended to be used in conjunction with standard of care culture for organism recovery, ...?"

A. We call and recommend that a bacterial culture be performed on the CSF if the test has not been ordered, but if the provider refuses we do not escalate the issue.

B. We insist that a bacterial culture must be performed on all CSF with orders for the M/E panel.

Does a bacterial culture need to be performed with every CSF specimen that is going to be tested with the BioFire M/E Panel?
For those who use the BioFire M/E panel, do you think there should be restrictions on the utilization of the panel?

A. Yes, only CSF specimens with distinctly abnormal cell counts and chemistries suggestive of meningitis should be tested.

B. No, the provider has made the decision to do a lumbar puncture and they must have meningitis in their differential since they are ordering the test.

Should restrictions be placed on the utilization BioFire M/E Panel?
Key Points - BioFire M/E Panel

1. Be aware of the organisms commonly associated with meningitis in the US.
2. The M/E panel targets some viruses where interpretation of a positive result can be challenging.
3. The assay is very sensitive and many of the M/E panel targets are commonly cultured, or handled, in the clinical microbiology laboratory.
4. Very strict adherence to good molecular testing techniques is required for reliable M/E panel performance.
5. Additional specimen collection, specimen handling, and workflow safeguards, above what are routinely done for other molecular testing, may be required for successful M/E panel testing.