The Clinical Benefits of Rapid Multiplex PCR Testing

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The System
How the FilmArray Works

Sample Prep + Amplification + Detection
The System
How the FilmArray Works
The System
How the FilmArray Works

Sample Extraction & Preparation

1st Stage Multiplex PCR

2nd Stage PCR

Reagent Storage

Chemical Circuit Board
The System
How the FilmArray Works
The System
How the FilmArray Works

1st Stage
Multiplex PCR

2nd Stage
Multiplex PCR

Dilute 100x
Automated Protocol
Bladders inflate over blisters to move liquid
Pistons open and close the channels
Plungers deliver reagents
The System
How the FilmArray Works

Results Analysis
102 individual 2\textsuperscript{nd} stage PCR wells
Each well contains one reaction
Melt curves generated for each well
Clinical Benefits:

Gastrointestinal Panel (GI)
Gastrointestinal (GI) Panel

**Bacteria**
- Campylobacter (*jejuni, coli, and upsaliensis*)
- Clostridium difficile (Toxin A/B)
- Plesiomonas shigelloides
- Salmonella
- Vibrio (*parahaemolyticus, vulnificus, and cholerae*)
  - *Vibrio cholerae*
- Yersinia enterocolitica

**Parasites**
- Cryptosporidium
- Cyclospora cayetanensis
- Entamoeba histolytica
- Giardia lamblia

**Diarrheagenic E. coli/Shigella**
- Enteroaggregative *E. coli* (EAEC)
- Enteropathogenic *E. coli* (EPEC)
- Enterotoxigenic *E. coli* (ETEC)
- Shiga-like toxin-producing *E. coli* (STEC)
  - *E. coli* O157
- Shigella/Enteroinvasive *E. coli* (EIEC)

**Viruses**
- Adenovirus F 40/41
- Astro virus
- Norovirus GI/GII
- Rotavirus A
- Sapovirus (I, II, IV, and V)

FDA-cleared for the first time.
Gastrointestinal Infections: Mortality and Costs

- 211–375 million episodes of diarrheal illness occur in the United States annually, resulting in:
  - 73,000,000 physician consultations
  - 1,800,000 hospitalizations
  - 3,100 deaths
  - $6 billion spent on medical care and lost productivity

The Current State of GI Testing

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Stool Culture</th>
<th>O&amp;P Staining</th>
<th>EIA</th>
<th>DFA</th>
<th>Traditional PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>3–5 days&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1–7 days&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>&lt;2 hours&lt;sup&gt;4&lt;/sup&gt;</td>
<td>30 mins&lt;sup&gt;5&lt;/sup&gt;</td>
<td>5–6 hours&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Parasites</td>
<td></td>
<td></td>
<td>Bacteria, Viruses, Parasites</td>
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<td>Bacteria, Viruses, Parasites</td>
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<tr>
<td>Time to Result</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>77%–91%&lt;sup&gt;7&lt;/sup&gt;</td>
<td>50%–90%&lt;sup&gt;8,9a&lt;/sup&gt;</td>
<td>75%–95%&lt;sup&gt;10&lt;/sup&gt;</td>
<td>90%–99%&lt;sup&gt;9&lt;/sup&gt;</td>
<td>up to 100%&lt;sup&gt;11&lt;/sup&gt;</td>
</tr>
<tr>
<td>Specificity</td>
<td>61%–78%&lt;sup&gt;7&lt;/sup&gt;</td>
<td>80%–90%&lt;sup&gt;9&lt;/sup&gt;</td>
<td>83%–98%&lt;sup&gt;10&lt;/sup&gt;</td>
<td>95%–100%&lt;sup&gt;9&lt;/sup&gt;</td>
<td>up to 100%&lt;sup&gt;11&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

DFA=direct fluorescent antibody; EIA=enzyme immunoassay; O&P=ova and parasite; PCR=polymerase chain reaction.

<sup>a</sup> Sensitivity scores are affected by a number of variables, including the number and quality of samples tested, previous antibiotic administration, and the proficiency of laboratory technicians.

Rapid Multiplex PCR Gastrointestinal Panel Can Enhance Pathogen Identification (ID) and Improve Hospital Infection Prevention (IP) Practices in Pediatric Patients with Diarrhea

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St. Christopher’s Hospital for Children4 and Drexel University College of Medicine6, Philadelphia, PA

**Background**

- Gastroenteritis accounts for >450,000 US hospitalizations annually; etiology is found in <50%.
- Multiplex PCR could provide rapid, simultaneous ID of multiple pathogens not suspected clinically or not detectable by standard tests (ST).
- Enhanced ID could impact IP practices.
- There are few studies of stool PCR testing in children with diarrhea.

**Results**

- 51 patients had 53 unique stool specimens analyzed.
- Majority of patients were hospitalized (91%).
- Median age was 4 years (range 10 days-17 years).
- 55% had chronic medical conditions.
- Diarrhea predominantly had community onset (CO-85%).
- Median of 3 (range 1-7) ST ordered/stool specimen.

Of 40 hospitalized patients with CO-diarrhea, only 85% were placed in enteric isolation.

Of 8 hospitalized patients with hospital-associated diarrhea, C. difficile was identified in 3 patients (both PCR and ST positive); only one out of these 3 patients with C. difficile diarrhea was placed in high-level enteric isolation.

**Objective**

- Compare BioFire FilmArray® GI Panel with standard tests (ST) for pathogen ID.
- Compare diagnostic yield of physician selected ST versus nonselective GI Panel.
- Assess the impact of rapid, enhanced ID on IP practices.

**Methods**

- Convenience sample of liquid stool specimens submitted to clinical lab for ST from Mar ’14-Mar ’15 were studied.
- ST performed per physician orders (ST could include stool culture, parasite microscopy, and enzyme immunoassays (EIA) for rotavirus, adenovirus, C. difficile (Wribosomal NAAT), Shigellae-like toxin-producing E. coli (STEC), Cryptosporidium, Giardia).
- GI PCR Panel performed as validation study, with one specimen test per patient per encounter, results not available in real time. GI panel tests for 22 pathogens (13 bacteria, 5 viruses, 4 protozoa).
- Medical charts reviewed retrospectively to assess clinical data and isolation measures.

PCR identified pathogens in 62% of specimens vs 30% for ST (p <0.005). PCR identified 23 additional pathogens among 17 ST-negative specimens (Fig 1).

**Conclusions**

- PCR enhanced pathogen ID >2 fold.
- Use of PCR could optimize isolation practices.
- Low yield of ST is due both to insensitivity and to inadequate physician selection of tests.
Challenges in Diagnosing Gastrointestinal Infections

- Limited clinical guidelines for the diagnosis and treatment of patients with suspected infectious diarrhea\(^1\)

- Challenges associated with currently available testing methods\(^1-4\):
  - Time-consuming
  - Labor-intensive
  - Technically complex/require specific expertise
  - Low yield
  - Lack sensitivity and specificity
  - Limited coverage
  - Confounded by:
    - Overlapping symptomology
    - Need to order multiple tests specific for suspected organisms
    - Unavailability of tests for many organisms

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Consequences of Misdiagnosis and Mistreatment of GI Infections

Potential outcomes of incorrect diagnosis and treatment

- Worsened illness\(^1\)
- Postinfectious sequelae\(^{1,2}\)
- Unnecessary side effects\(^{3,4}\)
- Antibiotic resistance\(^{3,5}\)

Early diagnosis facilitates timely and appropriate therapeutic interventions that can alleviate symptoms and prevent secondary transmission\(^1\)

Potential Patient and Provider Benefits

- Rapid diagnosis of the causative agent of GI infections and appropriate treatment decisions can improve patient outcomes and decrease healthcare costs\(^1,2\)

**Provider**
- Provides more comprehensive testing\(^4\)
- Informs improved quality of care\(^2\)
- Guides appropriate follow-up\(^3\)

**Patient**
- Shortened illness\(^1\)
- Shorter hospital visits\(^2\)
- Reduced morbidity\(^1\)
- Prevents secondary transmission\(^1\)

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Fast results\(^3\)
Comprehensive coverage\(^3\)
Accurate pathogen identification\(^3\)

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3. FilmArray GI [Instruction Booklet]. Salt Lake City, UT: BioFire Diagnostics, LLC.
Clinical Benefits: Blood Culture Identification Panel (BCID)
Blood Culture Identification (BCID) Panel

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

**Gram- Bacteria**
- Acinetobacter baumannii
- Haemophilus influenzae
- Neisseria meningitidis
- Pseudomonas aeruginosa
- Enterobacteriaceae
  - Enterobacter cloacae complex
  - Escherichia coli
  - Klebsiella oxytoca
  - Klebsiella pneumoniae
  - Proteus
  - Serratia marcescens

**Yeast**
- Candida albicans
- Candida glabrata
- Candida krusei
- Candida parapsilosis
- Candida tropicalis

**Antibiotic Resistance**
- mecA – methicillin resistant
- van A/B – vancomycin resistant
- KPC – carbapenem resistant

FDA-cleared for the first time.
Definitive identification of a pathogen can take 24 to 72 hours through traditional culture methods.

This delay can lead to inadequate or overly broad antimicrobial therapy and result in therapy-related complications, antimicrobial resistance, and increases in patient morbidity, mortality, and costs.

Unmet Needs in Treating Sepsis

A retrospective cohort analysis of 760 patients with severe sepsis¹

31% received inappropriate antibiotic treatment

In 58%, therapy was delayed

42% had resistance to the antibiotic administered

Patients who progress to septic shock have a 7.6% increase in mortality every hour while not on appropriate therapy.²

Septicemia remains a leading cause of death in both adults and infants in the United States, and is the leading cause of death in noncardiac ICUs\textsuperscript{1,2}.

- **Sepsis\textsuperscript{2}**: >1.1 million cases annually
- **Mortality\textsuperscript{2}**: >40%
- **$24.3 billion\textsuperscript{2}**: annual cost

ICU=intensive care unit.
A Fast Diagnosis Can Ensure Timely Treatment, Which May Reduce Mortality

Mortality Rate of Sepsis, Severe Sepsis, and Septic Shock

- Infection/sepsis: 26%
- Severe sepsis: 42%
- Septic shock: 61%

Timely treatment is essential to prevent the progression of sepsis to septic shock and reduce mortality¹⁻³

Clinical Benefits: Respiratory Panel (RP)
Respiratory Panel (RP)

**Viruses**
- Adenovirus
- Coronavirus HKU1
- Coronavirus NL63
- Coronavirus 229E
- Coronavirus OC43
- Human Metapneumovirus
- Human Rhinovirus/Enterovirus
- Influenza A
- Influenza A/H1
- Influenza A/H3
- Influenza A/H1-2009
- Influenza B
- Parainfluenza 1
- Parainfluenza 2
- Parainfluenza 3
- Parainfluenza 4
- Respiratory Syncytial Virus

**Bacteria**
- *Bordetella pertussis*
- *Chlamyophila pneumoniae*
- *Mycoplasma pneumoniae*

FDA-cleared for the first time.
Clinical and Economic Consequences of Respiratory Infections In the United States

- 25,000,000 family physician consultations¹
- 1,026,476 hospitalizations due to upper respiratory tract infections between 1998 and 2006²
- $40 billion estimated annual cost of non-influenza–related viral respiratory tract infections³

Unmet Needs in Diagnosing and Treating Respiratory Infections

Of influenza-positive children

43% were hospitalized within two days of symptom onset

but only 1.5% received antiviral treatment\(^1\)

Of outpatients with confirmed RSV infection

3% received a specific diagnosis of RSV infection\(^2\)

RSV=respiratory syncytial virus.

Clinical Benefits of Rapid and Accurate Diagnosis

- Rapid identification of the causative agent of respiratory infections can improve patient management by:
  - Informing timely and effective antibiotic or antiviral therapy
  - Preventing secondary spread of infection
  - Shortening hospital stays
  - Reducing costs of unnecessary tests

RP=respiratory panel.
Clinical Benefits: Meningitis/Encephalitis Panel (ME)
The Clinical Benefits of the ME Panel
How can rapid results impact your diagnosis?
Meningitis/Encephalitis (ME) Panel:

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

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

**Fungi**
- *Cryptococcus neoformans/gattii*
**IDSA Guidelines for the Management of Adults With Bacterial Meningitis (2004)**

1. **Suspicion for bacterial meningitis**

   - Immunocompromised, history of CNS disease, new onset seizure, papilledema, altered consciousness, or focal neurologic deficit; or delay in performance of diagnostic lumbar puncture
     - **No**
     - Blood cultures and lumbar puncture STAT
       - **Yes**
       - Dexamethasone\(^a\) + empirical antimicrobial therapy\(^b\)
         - **Yes**
         - CSF findings c/w bacterial meningitis
           - **Yes**
           - Positive CSF Gram stain
             - **No**
             - Dexamethasone\(^a\) + empirical antimicrobial therapy
               - **Yes**
               - Blood cultures STAT
                 - **Yes**
                 - Dexamethasone\(^a\) + empirical antimicrobial therapy
                   - **Yes**
                   - Negative CT scan of the head
                     - **Yes**
                     - Perform lumbar puncture
                       - **Yes**
                       - Dexamethasone\(^a\) + targeted antimicrobial therapy

2. **Lumbar puncture may be delayed in a number of clinical settings, including to permit CT of patients at high risk of raised ICP**\(^1\)
   - This includes patients such as Nina with new onset seizure

3. **In such cases, antimicrobial therapy should be initiated before lumbar puncture or CT scan**\(^1\)
   - In patients with suspected bacterial meningitis, 2 to 6 hour delays in therapy are associated with adverse outcomes\(^2\)

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\(^a\) Refer to specific recommendations for the use of adjunctive dexamethasone in adults with bacterial meningitis.

\(^b\) Dexamethasone and antimicrobial therapy should be administered immediately after CSF is obtained.

CNS=central nervous system; CSF=cerebrospinal fluid; CT=computed tomography; ICP=intracranial pressure; IDSA=Infectious Diseases Society of America.

# Negative Results Do Not Always Equal No Infection

Administering empiric antibiotic therapy prior to CSF collection can confound traditional diagnoses and present therapeutic challenges\(^1,2\)

<table>
<thead>
<tr>
<th>Traditional Diagnostic Techniques</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CSF culture:</strong> antibiotic therapy can reduce bacterial load to undetectable levels within 1 hour(^1)</td>
<td><strong>Performance is less affected by prior antibiotic therapy(^5)</strong></td>
</tr>
<tr>
<td><strong>Gram stain:</strong> sensitivity may be attenuated by prior antibiotic therapy(^3)</td>
<td></td>
</tr>
<tr>
<td>• Negative Gram stains may suggest viral meningitis, but do not rule out bacterial meningitis or other urgent treatable causes(^4)</td>
<td></td>
</tr>
<tr>
<td>• Uncertainty may result in unnecessary empiric antibiotic and antiviral therapy</td>
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</tr>
</tbody>
</table>

PCR provides more rapid detection and enhanced sensitivity, guiding timely and appropriate patient management\(^5\)

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CSF=cerebrospinal fluid; ME=meningitis/encephalitis; PCR=polymerase chain reaction.

A Fast and Accurate Diagnosis Can Improve Treatment Outcomes

Benefits of a fast and accurate diagnosis of bacterial meningitis include\(^1,^2\):

- Reduced mortality and adverse outcomes\(^1\)
- Specific therapy administered in a timely manner\(^2\)
- Infection control precaution implementation and chemoprophylaxis to prevent spread of infection\(^2\)
- Decreased costs associated with inappropriate therapies and adverse outcomes\(^2\)

- For every hour delay in antibiotic therapy, the odds for adverse outcomes of bacterial meningitis may increase by up to 30\(^%\)^3

Rapid diagnosis of *N meningitidis* and rapid administration of appropriate therapy to Nina may reduce her risk of developing adverse outcomes

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Patients with suspected viral meningitis should be treated as if they are infected with bacterial meningitis until a bacterial etiology has been excluded, at which point antibiotic therapy should be discontinued\(^1\)

- Antiviral therapy is not available for the majority of viral agents causing meningitis, including enterovirus\(^2,3\)

Most patients recover completely within 7–10 days of disease onset; however, complications such as seizures and coma occur in ~10\% of cases\(^2,4\)

Supportive therapy should be provided to appropriately manage patients\(^3\)

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PCR=polymerase chain reaction.

Early detection of enteroviral meningitis with PCR can improve patient care and afford significant cost savings by reducing the duration of unnecessary hospitalizations and parenteral antibiotics. In a retrospective cohort study of children with meningitis, PCR decreased the time to enteroviral diagnosis from 53 hours to 12 hours.

For every hour saved, length of stay and duration of parenteral antibiotics decreased by 0.3 hours.

Median hospital stay decreased from 44 to 28 hours.

Median duration of parenteral antibiotics decreased from 48 to 36 hours.

PCR = polymerase chain reaction.
CNS fungal infections require prompt and precise diagnosis due to their high risks of morbidity and mortality\(^1\)

Appropriate medical and/or surgical management strategies should be implemented promptly to achieve successful patient outcomes\(^1\)

Specific induction, consolidation, and suppressive treatment regimens are recommended for *Cryptococcus* infection\(^2\)

Specific complications associated with *Cryptococcus* include immune reconstitution inflammatory syndrome, increased intracranial pressure, and cryptococcomas. These should be monitored and managed as necessary\(^2,3\)

CSF=cerebrospinal fluid; CNS=central nervous system; ME=meningitis/encephalitis.

PCR Is The Gold Standard for Encephalitis Diagnosis

IDSA guidelines recommend that HSV PCR should be performed on all CSF specimens in patients with encephalitis

PCR is considered to be the gold standard in diagnosing viral agents of encephalitis

- Extremely high sensitivity and specificity
- Positive early in course of disease
- Detects atypical forms of HSV-1 encephalitis previously attributed to other viral agents
- Eliminates the need for brain biopsy

CSF=cerebrospinal fluid; HSV-1=herpes simplex virus-1; IDSA=Infectious Diseases Society of America; ME=meningitis/encephalitis; PCR=polymerase chain reaction.

Benefits of Early Diagnosis and Therapy on Encephalitis Outcomes

Rapid and accurate diagnosis of HSV-1 infection can help achieve prompt initiation of appropriate therapy.

IV acyclovir should be administered early\(^1\)

Early, aggressive antiviral therapy can prevent mortality\(^1\)

Prompt therapy can limit the severity of chronic behavioral and cognitive impairments\(^1,2\)

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HSV-1=herpes simplex virus-1; IV=intravenous.

Questions?