DETECTION OF VIRA LiAL DIARRHEAL PATHOGENS BY THE FILMARRAY® GI POUCH

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FILMARRAY GI PANEL
An estimated 211-335 million episodes of diarrheal illnesses occur each year in the United States, resulting in 72 million physician consultations, 1.8 million hospitalizations, and 3,000 deaths. The current challenge is diagnosing infectious diarrheas in the large number of patients that are known to cause diarrhea, including viruses, bacteria, and protozoa. Present diagnostic practice is insufficient to meet multiple needs which are labor-intensive, expensive, and slow. Additionally, many gastrointestinal (GI) pathogens have limited test availability and/or low sensitivity. There is a clear unmet clinical need for a rapid, easy-to-use, multi-target, and highly sensitive molecular diagnostic test for the detection of pathogens causing diarrhea.

IT is developing a new FilmArray panel for detection of GI pathogens (FA GI panel) from stool specimens of patients presenting with diarrhea. The FA GI panel targets 28 pathogens including viruses, bacteria, and protozoa. Testing requires minimal pre-processing of specimens. The test is a 1:10 dilution in Cary-Blair, filtered by gravity-flow through a coarse strainer, and loaded into the FilmArray GI pouch using a novel fiber-insertion rail. An automated report listing all pathogens detected is ready in about an hour. A preliminary evaluation of both specificity and sensitivity of viral detection by the FA GI panel is presented. Performance of the viral assays in the FA GI panel was performed on pediatric clinical specimens.

Currently Targeted Organisms

<table>
<thead>
<tr>
<th>Organism/Strain</th>
<th>Viral Detection</th>
<th>Bacterial Detection</th>
<th>Protozoal Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus (F40, F41)</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Adenovirus (B7d/d2, C2-1, C6, F40)</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Adenovirus (B14, C10)</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

SPECIFICITY
The ability of the FilmArray GI pouch to detect a broad range of enteroic viruses were assessed on characterized strains or clinical isolates obtained from viral collections.

The Adenovirus assay specifically detected both serotypes F40 and F41 while proving negative for 16 strains of adenovirus A, B, C, D, and E. It is expected to exclude adenovirus G as well.

The Human Astrovirus (HAstV) assay is designed to detect 1-8 but not novel strains of norovirus MLV, GI1, GI2, GI4, GI5, nor VA4, for which clinical relevance is still under investigation. Detection of new norovirus genotypes 1, 4, 6, and 7 has been confirmed. The FA GI pouch detected types 1, 4, and 6 but not strains MLV and VA2. The remaining serotypes will be tested as they become available.

The Norovirus assays were designed to detect all strains of Norovirus that cause illnesses in humans, which include Norovirus GI-1, GI-2, GI-3, and GI-4. The GI-5 was not tested.

The Rotavirus assays were designed to be as inclusive as possible for all human serotypes of Rotavirus A, B, C, and D. The FilmArray GI pouch successfully detects Rotavirus A strains 1, 2, 3, and 4, and synthetic RNA based on Rotavirus B D1-1 and Rotavirus C Mu2. Others were not tested.

The Sapovirus assay was designed to detect all genotypes known to cause diarrheas in humans which include Genogroup I, 2, 3, and 4. Detection of all Norovirus which cause illnesses in humans were confirmed by the FilmArray GI pouch. The detection of each virus was shown to be specific. No cross-reactivity with other pathogens included in the FA GI panel was observed.

The FilmArray test is initiated by injecting rehydration solution and a target sample into the FilmArray pouch and placing it into the FilmArray instrument. The user selects the sample and pouch type (using a barcode reader) into the software and initiates the run. Results are provided in ~1 hour.

The FilmArray pouch has a filter (see label A) containing all needed freeze-dried reagents.

Analytical Testing - Sensitivity

<table>
<thead>
<tr>
<th>Organism/Strain</th>
<th>Quantity Detected</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus (F40)</td>
<td>10.50 TCID50/mL</td>
<td>ZeptoMetrix</td>
</tr>
<tr>
<td>Adenovirus (B)</td>
<td>2.19 TCID50/mL</td>
<td>ZeptoMetrix</td>
</tr>
<tr>
<td>Adenovirus (B14)</td>
<td>1.70 TCID50/mL</td>
<td>ZeptoMetrix</td>
</tr>
<tr>
<td>Adenovirus (C10)</td>
<td>1.14 TCID50/mL</td>
<td>ZeptoMetrix</td>
</tr>
<tr>
<td>Rotavirus (A)</td>
<td>2.42 TCID50/mL</td>
<td>ZeptoMetrix</td>
</tr>
<tr>
<td>Rotavirus (B)</td>
<td>3.02 TCID50/mL</td>
<td>ZeptoMetrix</td>
</tr>
<tr>
<td>Rotavirus (C)</td>
<td>2.01 TCID50/mL</td>
<td>ZeptoMetrix</td>
</tr>
<tr>
<td>Rotavirus (D)</td>
<td>1.97 TCID50/mL</td>
<td>ZeptoMetrix</td>
</tr>
</tbody>
</table>

A. Denaturation
B. Magnetic solid-phase nucleic acid extraction
C. Cell lysis
D. Wash station
E. Magnetic bead collection blister
F. Reagent-1 blister
G. Multiplex Outer PCR blister
H. Catalyse solution blister
I. Yersinia enterocolitica
J. Porcine norovirus
K. Norovirus G4_#8
L. Norovirus G4_#12
M. Norovirus G4_#13

Quantitated Saposivus was unavailable at time of testing.
Rotavirus B and Rotavirus C were detected at 100 copies of synthetic RNA/pouch.

Performance of FilmArray assays vs comparator’s RTPCR assays

The ability of the individual FilmArray assays to detect a broad range of Noro and Sapovirus strains was confirmed by testing an extensive collection of RNAs extracted from fecal specimens and characterized as specific strains of Noro or Sapovirus by the providing lab. The FA GI assays were able to detect all strains shown below.

REFERENCES

CONTACT INFORMATION
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