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Comparison of three multiplex gastrointestinal platforms for the detection of gastroenteritis viruses

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ABSTRACT

Background: Viruses are major etiological agents of childhood gastroenteritis. In recent years, several molecular platforms for the detection of viral enteric pathogens have become available.
Objective/study design: We evaluated the performance of three multiplex platforms including Biofire's Gastrointestinal Panel (FilmArray), Luminex xTAG® Gastrointestinal Pathogen Panel (GPP), and the TaqMan Array Card (TAC) for the detection of five gastroenteritis viruses using a coded panel of 300 archived stool samples.
Results: The FilmArray detected a virus in 199 (96.1%) and the TAC in 172 (83.1%) of the 207 samples (187 samples positive for a single virus and 20 samples positive for more than one virus) whereas the GPP detected a virus in 100 (78.7%) of the 127 (97 positive for one virus and three positive for more than one virus) samples. Overall the clinical accuracy was highest for the FilmArray (98%) followed by TAC (97.2%) and GPP (96.9%). The sensitivity of the FilmArray, GPP and TAC platforms was highest for rotavirus (100%, 95.8%, and 89.6%, respectively) and lowest for adenovirus type 40/41 (97.4%, 57.9% and 68.4%). The specificity of the three platforms ranged from 95.6% (rotavirus) to 99.6% (norovirus/sapovirus) for the FilmArray, 99.6% (norovirus) to 100% (rotavirus/adenovirus) for GPP, and 98.9% (astrovirus) to 100% (rotavirus/sapovirus) for TAC.
Conclusion: The FilmArray demonstrated the best analytical performance followed by TAC. In recent years, the availability of multi-enteric molecular testing platforms has increased significantly and our data highlight the strengths and weaknesses of these platforms.

1. Background

Acute gastroenteritis is an important public health burden causing nearly 2 million cases of diarrheal disease each year globally [1]. The disease has been associated with a diverse group of etiologic agents, which includes bacteria, viruses and parasites [2]. Rotavirus, norovirus, adenovirus types 40 and 41, astrovirus, and sapovirus are the five major viral agents of acute gastroenteritis in humans accounting for nearly 60% of the medically-attended childhood gastroenteritis in the United States [3]. Before the introduction of rotavirus vaccines in the United States, nearly all children were infected with rotavirus before age 5 [4]. After the decline of rotavirus as the results of rotavirus vaccination, noroviruses have become the leading cause of acute gastroenteritis in the United States, especially in children < 5 years of age [5–8].

Different laboratory methods such as viral antigen detection using enzyme immunoassays or immunochromatographic assays or molecular methods such as conventional (RT-)PCR and real-time (RT-)PCR assays have been employed by clinical laboratories to test for gastroenteritis viruses. Some of these methods are time-consuming and most of them usually test for only one virus per assay [9]. In comparison, multiplex molecular assays for the simultaneous detection of known gastrointestinal pathogens, including viruses, reduces turnaround time for accurate results and also identify infections and/or co-infections that remained undiagnosed by routine test methods for single pathogens. In

Abbreviations: GPP, Gastrointestinal Pathogen Panel; TAC, TaqMan Array Card

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this study, we evaluated the performance characteristics of three multiplex platforms for the detection of gastroenteritis viruses. These included the BioFire gastrointestinal panel (FilmArray), the Luminex xTag GI pathogen panel (GPP) and the TaqMan Array Card (TAC) system. These platform were chosen because they are FDA cleared (FilmArray and GPP) or available for large throughput testing (TAC).

The FilmArray is a cartridge-based integrated system which combines automated sample preparation, nucleic acid extraction and multiplex PCR-based detection using DNA melting curve analysis [10]. Luminex GPP (US-IVD) is based on multiplex RT-PCR for target amplification and detection using Luminex microsphere xMAP and xTAG technologies [10]. The TAC system is custom developed by Life Technologies and contains singleplex or duplex real-time PCR reactions, allowing for multi-target detection through spatial distribution [11].

2. Objective

To evaluate the performance of three multiplex gastrointestinal platforms, a panel of coded stool samples including five different gastroenteritis viruses was compiled and shipped to three study sites each of which tested the panel using one of the three platforms and results were compared to reference methods for each of the individual viruses.

3. Study design

A panel of 300 stool samples was compiled at CDC and included 187 samples positive for one of the five viruses (rotavirus, norovirus, sapovirus astrovirus and adenovirus 40/41), 20 samples positive for at least two viruses by TaqMan realtime (RT-) PCR and conventional (RT-) PCR followed by Sanger sequencing [12–20], and 93 samples negative for any of these viruses. The stool samples were collected from norovirus outbreaks (children and adults) that occurred between 2008 and 2012 [21] and from sporadic cases (children) of acute gastroenteritis [6,3]. Stool samples had been stored at −70 °C. The panel included 48 samples positive for rotavirus, 41 samples positive for norovirus (GI and/or GII), 41 samples positive for sapovirus, 39 samples positive for astrovirus and 38 samples positive for adenovirus 40/41. The panel included genotypes of each virus circulating in the United States (Table 1). The panel was coded and distributed to three New Vaccine Surveillance Network (NVSN) [22] study sites (Kansas City, MO. Nashville, TN, and Rochester, NY) and one panel was kept at CDC. At CDC, the panel was retested using both reference methods described above by a different laboratorian than who had compiled the panel. At the Children's Mercy Hospital in Kansas City, MO the panel was tested on the FilmArray, at Vanderbilt University Medical Center, Nashville, TN on Luminex GPP; and at Golisano Children's Hospital in Rochester, NY on the TAC system. Institutional review approval for the parent NVSN protocols was granted by CDC and each participating site's Institutional Review Boards.

The FilmArray and TAC systems are able to detect all five viruses (adenovirus 40/41, astrovirus, norovirus, rotavirus and sapovirus) whereas Luminex GPP detects three viruses (adenovirus 40/41, norovirus and rotavirus) (Table 2). In addition to the viruses, the FilmArray is able to detect 13 bacterial and four parasite organisms; Luminex GPP is able to detect 8 bacterial and 3 parasite organisms; and the TAC system employed in this study could detect 15 enteric bacterial, 3 protozoal, 10 parasite, and 2 fungal organisms as well as enteroviruses (Table 2).

Table 1
Virus genotypes included in stool panel to compare performance of three different enteric pathogen platforms.

Virus	Genotypes
Adenovirus	type 41
Astrovirus	type 1–5 and 8
GI Norovirus	GI.1. GI.3, GI.4, GI.5, GI.6, GI.8 and GI.9
GII Norovirus	GII.2, GII.3, GII.4 New Orleans, GII.4 Sydney, GII.6, GII.7, GII.8, GII.12, GII.13, GII,14, GII.16, GII.17
Rotavirus	G2P[4], G3P[8], G9P[8], G12P[8]
Sapovirus	GI.1. GI.2, GI.3, GII.1, GII.2, GII.3, GII.5, GII.6, GIV and GV

Testing stool samples on the FilmArray was performed according to the manufacturer's instructions [10] which requires approximately 5 min of hands-on time and results are available in 1 h (Table 3). Briefly, 200 µL of the stool mixed in sample buffer (provided by the manufacturer) was added to the sample injection port. The FilmArray pouch was rehydrated with hydration solution and then inserted into the instrument (Biofire Diagnostics, Salt Lake City, UT, USA). Each pouch contained an internal nucleic acid extraction control and a PCR control. Runs where the internal controls failed were considered invalid for all panel analytes and those specimens were retested.

Testing on Luminex GPP was performed in accordance with the manufacturer's instructions [10] and takes approximately 60 min of hands-on time and results are available within 6 h (Table 3). Briefly, 100 µL of 10% clarified stool suspension prepared in phosphate buffered saline was combined with 10 µL xTAG® MS2 and pre-treated by vortexing in a Bertin SK38 bead tube containing 1 mL bioMérieux® NucliSENS® easyMAG® Lysis Buffer. Nucleic acid was extracted by using the NucliSENS easyMAG system (BioMerieux, NC, USA). PCR reactions and subsequent hybridization step were performed according to the manufacturer's instructions. The data were acquired on the Luminex 200 analyzer, and data analysis was carried out using TDAS data analysis software.

The TAC system can process and analyze up to six samples at a time for up to 40 pathogens tested; and requires approximately 60 min of hand-on time and 3 h to obtain results (Table 3). Nucleic acid was extracted from 200 mg of stool lysed with QiaAmp ASL buffer and homogenized using a bead beater [11] followed by QiaAmp nucleic acid extraction protocol (Qiagen, Valencia, CA). TAC assays were designed as described previously [11] and purchased from Eric R. Houpt (University of Virginia). Ag-Path-ID one step RT-PCR kit (Applied Biosystems, Carlsbad, CA, USA) was used and the reaction mixture was loaded into each port of the card after which the card was briefly centrifuged twice at 1200 rpm for 1 min. The card was then sealed, the loading ports excised, and run on a ViiA7 real-time PCR (Applied Biosystems-Thermo Fisher). The reaction conditions consisted of reverse transcription at 45°C for 20 min followed by 10 min at 95°C to activate the Taq polymerase, 45 PCR cycles at 95°C for 15 s and 60°C for 1 min.

Samples were considered true positive for a virus when they tested positive by both reference testing methods (i.e. (RT-)qPCR and sequencing) and samples negative in both methods were considered true negative. We used a cycle threshold (Ct) values of 40 as cut-off for the TaqMan real-time assays. The percent clinical accuracy, sensitivity and specificity for each of the five viruses for each platform were calculated as follows:

% clinical accuracy: 100 X (true positives + true negatives)/(true positives + true negatives + false positive + false negative).

% sensitivity: 100 X (true positives/(true positives + false negatives))

% specificity: 100 X (true negatives/(true negatives + false positives))

4. Results

Among the 207 true virus-positive samples (187 samples positive for a single virus and 20 samples positive for more than one virus), the FilmArray detected 199 (96.1%) samples and the TAC system detected 172 (83.1%) samples (Table 4). The GPP, which is only able to detect rotavirus, adenovirus type 40/41 and norovirus, detected 100 (78.7%) of the 127 true virus-positive samples positive for one or more of the three gastroenteritis viruses. No correlation was found between number of samples missed by each platform and virus genotypes. The overall