Comparative Evaluation of Seegene Allplex Gastrointestinal, Luminex xTAG Gastrointestinal Pathogen Panel, and BD MAX Enteric Assays for Detection of Gastrointestinal Pathogens in Clinical Stool Specimens

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Context.—Infectious gastroenteritis is caused by various pathogens, including bacteria, viruses, and parasites.

Objective.—To compare the performance of Seegene Allplex Gastrointestinal (24 targets: 13 bacteria, 5 viruses, and 6 parasites in 4 panels), Luminex xTAG Gastrointestinal Pathogen Panel (15 targets: 9 bacteria, 3 viruses, and 3 parasites), and BD MAX Enteric panel (5 bacteria and 3 parasites). We estimated the agreement among 3 molecular assays.

Design.—A total of 858 stool samples (554 bacterial/parasite and 304 viral pathogens) were included. A consensus positive/negative was defined as concordant results from at least 2 tests. To evaluate the agreement among the assays, \( \kappa \) value was calculated.

Results.—The overall positive percentage agreements of Seegene, Luminex, and BD MAX were 94% (258 of 275), 92% (254 of 275), and 78% (46 of 59), respectively. For Salmonella, Luminex showed low negative percentage agreement because of frequent false positives (n = 31) showing low median fluorescent intensity. For viruses, positive/negative percentage agreements of Seegene and Luminex were 99%/96% and 93%/99%, respectively. Compared with routine microbiology testing, Seegene, Luminex, and BD MAX additionally identified 39, 40, and 12 pathogens, respectively. Sixty-one cases (16 cases with Seegene, 51 cases with Luminex, and 1 case with BD MAX) showed positive results for multiple pathogens, but only 3 were consensus positive.

Conclusions.—These multiplex molecular assays appear to be promising tools for the detection and identification of multiple gastrointestinal pathogens simultaneously. However, careful interpretation of positive results for multiple pathogens is required.

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management, and infection control. Thus, the use of efficient methods for pathogen detection is necessary to ensure rapid turnaround time. For example, real-time PCR can detect *Salmonella* spp, *Shigella* spp, and *Campylobacter* spp with superior sensitivity to bacterial culture. In addition, various diarrheagenic *E. coli* strains cannot be identified using routine culture. As sensitivity and specificity of each method differ, the selection of detection method is key for efficient diagnosis. Most laboratories in Korea use EIA or immunochromatographic assay for viral pathogens and microscopic examination or EIA for parasites. Very recently, several commercial multiplex tests have been developed and Conformité Européenne In Vitro Diagnostic has approved them for the detection of enteric pathogens in stool specimens. The use of these commercial tests may have significant efficiencies in detection of enteric pathogens.

In this study, to estimate the agreement among 3 molecular assays, we compared the clinical performance of Seegene Allplex GI full panel (24 targets: 13 bacteria, 5 viruses, and 6 parasites in 4 panels; Seegene, Seoul, Korea), Luminex xTAG Gastrointestinal Pathogen Panel (GPP; 15 targets: 9 bacteria, 3 viruses, and 3 parasites; Luminex Corporation, Austin, Texas), and BD MAX Enteric panel (5 bacteria and 3 parasites, BD Diagnostics, Sparks, Maryland).

**MATERIALS AND METHODS**

**Clinical Samples and Routine Diagnostic Procedures**

A total of 858 stool samples submitted to the department of laboratory medicine in the hospital were collected between January and October 2016. The raw stool samples from all patients were collected and a single stool sample was obtained from each patient. Each sample was submitted for 1 of 4 different microbiological tests: 197 samples (23%) for in-house multiplex PCR assays for diarrheagenic *E. coli* and *Salmonella* spp; 182 samples (21%) for routine bacterial culture, 175 samples (20%) for toxigenic *Clostridium difficile* culture and Xpert *C. difficile* Epi (Cepheid, Sunnyvale, California), and 304 samples (36%) for norovirus and rotavirus antigen test. All 3 assays allowed the use of raw stool samples. The remaining samples were dispensed in aliquots and stored at −70°C until used. The number of positive samples in each pathogen was determined using routine culture and Xpert assay (Cepheid) was performed on the Xpert C difficile assay (Cepheid). Necrotic acid was extracted from stool samples using the QIAamp DNA Stool Mini Kit (Qiagen) and subjected to multiplex PCR for detecting the presence of diarrheagenic *E. coli* and *Salmonella* spp. The DNA templates were subjected to multiplex PCR with specific primers for the detection of the following virulence markers: *aggR* and *sepA* for enterogaegregative *E. coli* (EAEC), *sxt/l2* for STEC, *ipaH* for enteroinvasive *E. coli* (EIEC)/*Shigella* spp, *eaeA* and *escV* for enteropathogenic *E. coli* (EPEC), *elt* and *estb* for enterotoxigenic *E. coli* (ETEC), and *imxA* for *Salmonella* spp, along with *uidA* as internal control as previously described elsewhere.

**Seegene Allplex Gastrointestinal Full Panel Assay**

Necrotic acids were extracted using the Microlab Nimbus IVD system (Hamilton, Reno, Nevada). Briefly, 100 to 200 mg of stool specimens was picked with a swab and suspended in 1 mL of stool lysis buffer (ASL buffer; Qiagen, Valencia, California). After pulse vortexing for 1 minute and incubation at room temperature for 10 minutes, the tubes were centrifuged at full speed (14,000 rpm) for 2 minutes. The supernatant was used for necrotic acid extraction. The Microlab Nimbus IVD system automatically performed the necrotic acid processing and PCR setup. Fluorescence was detected at 2 temperatures (60°C and 72°C), and a positive test result was defined as a well-defined exponential fluorescence curve that crossed the crossing threshold at a value of less than 42 for individual targets.

**Luminex xTAG GPP Assay**

The stool samples (about 100 µL) were pretreated before necrotic acid extraction according to the manufacturer’s instructions. Briefly, an internal control (bacteriophage MS2) and stool samples were added to a Berkin SK 38 Soil Mix Bead tube (BioAmerica Inc, Miami, Florida) containing 900 µL of easyMAG lysis buffer (bioMérieux, Durham, North Carolina). Tubes were vortexed for 5 minutes and allowed to settle for 10 minutes at room temperature, then centrifuged at 5000 rpm for 5 minutes. The supernatant (200 µL) was used for necrotic acid extraction using the QIAamp MinElute Virus Spin kit (Qiagen, Valencia, California). For hybridization, a bead mixture was prepared by adding 100 µL of Luminex MagFlex-TAG microsphere set to a sterile polystyrene tube and was vortexed and sonicated. The supernatant was removed, and the beads were resuspended in xTAG buffer (Luminex Corporation) and dispensed in each well of a microplate. Then, the PCR product (5 µL) was added to the wells and was mixed by pipetting up and down. The plate was sealed and was allowed to hybridize in the thermocycler. Negative and positive controls were included in all runs. The mean fluorescence intensity (MFI) was generated for each bead population and analyzed automatically by the xTAG Data Analysis Software GPP version 1.11. Although Luminex is not designated as a quantitative assay, we compared MFI values between consensus-positive and false-positive cases.

**BD MAX Enteric Panel Assay**

A 10-µL loop was used to place the stool specimen into the BD MAX sample buffer tubes. After vortexing, the tubes were loaded into the BD MAX instrument along with the BD MAX enteric bacterial panel reagent strip. The automated process included the preparation of the sample, lysis and extraction of the necrotic acid, and the performance of a multiplex PCR assay.

**In-House Multiplex PCR Assay for Diarrheagenic *E. coli* and *Salmonella* spp**

Necrotic acid was extracted from stool samples using the QIAamp DNA Stool Mini Kit (Qiagen) and subjected to multiplex PCR for detecting the presence of diarrheagenic *E. coli* and *Salmonella* spp. The DNA templates were subjected to multiplex PCR with specific primers for the detection of the following virulence markers: *aggR* and *sepA* for enterogaegregative *E. coli* (EAEC), *sxt/l2* for STEC, *ipaH* for enteroinvasive *E. coli* (EIEC)/*Shigella* spp, *eaeA* and *escV* for enteropathogenic *E. coli* (EPEC), *elt* and *estb* for enterotoxigenic *E. coli* (ETEC), and *imxA* for *Salmonella* spp, along with *uidA* as internal control as previously described elsewhere.

**Xpert C difficile Assay**

The Xpert *C difficile* assay (Cepheid) was performed on the Cepheid GeneXpert Dx System. First, a swab was dipped into the stool specimen and it was placed in the sample reagent and capped. The specimen was vortexed for 10 seconds, and all the liquid was transferred to the S chamber of the cartridge using a transfer pipette.

**Norovirus and Rotavirus Antigen Test**

The immunochromatography test for norovirus antigen was performed using the QuickNavi-Norovirus2 kit (Denka Seiken Co, Ltd, Tokyo, Japan) according to the manufacturer’s directions. Rotavirus EIA was performed using an RIDASCREEN Rotavirus Test Kit (R-BioPharm, Darmstadt, Germany) in accordance with the methods recommended by the manufacturer. An automated
immunoassay system, GEMINI (Stratec Biomedical, Birkenfeld, Germany), was used.

**Comparative Evaluation**

A consensus positive was defined as a positive result from at least 2 tests, including routine diagnostic procedure. A consensus negative was defined as a negative result from at least 2 of the 3 assays as long as the organism was a target on the assays.

The targets included in 3 commercial assays are described in Table 1. Among the bacterial/parasite pathogens, the results of *Campylobacter* spp, *Salmonella* spp, *Shigella* spp, STEC, and *Cryptosporidium* spp, which were included in all 3 assays, were compared among the Seegene Allplex bacterial panel, Luminex xTAG GPP, and BD MAX Enteric bacterial panel. However, as the BD MAX Enteric bacterial panel does not contain a target for *C. difficile*, specimens positive for *C. difficile* by either Seegene or Luminex were confirmed by Xpert *C. difficile* assay (Cepheid). For EAE and EPEC, which are included only in Seegene Allplex and in-house PCR, the discrepant results were resolved by the FilmArray assay (bioMérieux).

In addition, as a virus detection kit is not available for BD MAX, the results of Seegene and Luminex assays were compared with an antigen test. In some cases, we were unable to arbitrate discordant test results because of the lack of an additional test method.

**Statistical Analyses**

As we defined consensus results as true-positive/true-negative instead of using a reference method, the estimates were called positive percentage agreement (PPA) and negative percentage agreement (NPA), rather than sensitivity and specificity. Positive percentage agreement is the number of positive samples from each method among the number of samples showing concordant positive from at least 2 methods. Negative percentage agreement is the number of negative samples from each method among the number of samples showing concordant negative from at least 2 methods. To evaluate the agreement among the assays, an interrater agreement statistic (κ) was calculated and interpreted according to Bland and Altman’s guidelines: less than 0 as indicating no agreement, 0 to 0.20 as slight, 0.21 to 0.40 as fair, 0.41 to 0.60 as moderate, 0.61 to 0.80 as substantial, and 0.81 to 1 as almost perfect agreement. The statistical differences in terms of MFI values between routine laboratory results and newly identified results were estimated by independent *t* test. All tests were 2-tailed, and *P* values were considered significant when less than .05. The statistical analyses including 95% CIs were performed using MedCalc version 17.6 (MedCalc software, Mariakerke, Belgium). A χ² test was used for comparisons of PPA and NPA for each method when the number of values in denominators for comparison was 5 or more. Otherwise, a Fisher exact test was performed.

**RESULTS**

A total of 275 GI pathogens (32%), including 42 *Campylobacter* spp, 72 *C. difficile*, 14 *Salmonella* spp, 3 *Shigella* spp, and 1 *Cryptosporidium* spp in bacterial/parasite pathogens and 63 norovirus, 79 rotavirus, and 1 adenovirus in viral pathogens, were detected in the 858 stool specimens (Table 2). Among them, 40 consensus positive GI pathogens were additionally identified compared with routine methods: 15 *Campylobacter* spp (9 by all 3 assays, 5 by Seegene and Luminex, and 1 by Luminex and BD MAX), 3 *Salmonella* spp (1 by all 3 assays, 2 by Seegene and Luminex), 2 *Shigella* spp (1 by all 3 assays, 1 by Seegene and Luminex), 18 *C. difficile* (by Seegene and Luminex), 1 *Cryptosporidium* spp (by Seegene and Luminex), and 1 adenovirus (by Seegene and Luminex). Among the 40 additionally detected pathogens, 29 (15 *C. difficile*, 11 *Campylobacter* spp, 2 *Shigella* spp, and 1 *Salmonella* spp) were among the 197 specimens submitted for in-house PCR for diarrheagenic *E. coli* and *Salmonella* spp and 9 (4 *Campylobacter* spp, 3 *C. difficile*, 1 *Salmonella* spp, and 1 *Cryptosporidium* spp) were among the 182 specimens submitted for bacterial culture. Only 1 *Salmonella* spp was additionally detected among the 175 specimens submitted for *C. difficile* assay, and 1 adenovirus was additionally detected among the 304 specimens submitted for norovirus/rotavirus assay.

The overall PPA of Seegene, Luminex, and BD MAX were 94% (258 of 275), 92% (254 of 275), and 78% (46 of 59), respectively. For pathogenic bacteria/parasite, false-positive/false-negative rates of Seegene, Luminex, and BD MAX were 2%/6% (12 of 583 and 17 of 275), 9%/7% (50 of 583 and 19 of 275), and 1%/23% (3 of 319 and 14 of 60), respectively. The denominators are adjusted according to the assay targets. The detailed results of the 3 assays are provided in Table 2.

Examining the bacterial species, for *Campylobacter* spp, a κ agreement of Seegene, Luminex, and BD MAX was 0.932 (95% CI, 0.873–0.991), 0.905 (95% CI, 0.840–0.970), and 0.831 (95% CI, 0.737–0.924), respectively. The PPA of Seegene, Luminex, and BD MAX was 88% (37 of 42), 100% (42 of 42), and 76% (32 of 42), respectively, and NPA was 100% (512 of 512), 98% (504 of 512), and 100% (510 of 512), respectively. For *Salmonella* spp, a κ agreement of Seegene, Luminex, and BD MAX was 0.823 (95% CI, 0.671–0.975), 0.454 (95% CI, 0.297–0.610), and 0.927 (95% CI, 0.826–1), respectively. The PPA of Seegene, Luminex, and BD MAX was 86% (12 of 14), 100% (14 of 14), and 93% (13 of 14), respectively, and the NPA was 99% (537 of 540), 94% (509 of 540), and 100% (540 of 540), respectively.

Luminex showed relatively lower κ value because of frequent false positives in *Salmonella* spp (n = 31), which revealed lower MFI (430; 95% CI, 137–723) of probe 1 than that of true-positive (1394; 95% CI, 1297–4086) (P < .001). For *C. difficile*, Seegene and Luminex showed almost perfect strength of agreement (0.908 [0.855–0.961] and 0.849 [0.783–0.915]) with equal PPA (88%) and NPA (100% and 98%). Two *Shigella* spp and 1 *Cryptosporidium* spp were detected by 2 or more assays and thus were regarded as consensus positive.

**Comparison of 3 Multiplex Assays and In-House PCR for Detecting Diarrheagenic *E. coli***

The results for diarrheagenic *E. coli* were compared only between Seegene and in-house PCR, because only some of the 6 pathotypes of diarrheagenic *E. coli* could be detected by Luminex (O157, ETEC, STEC) and BD MAX (STEC and EIEC). Twenty-four diarrheagenic *E. coli* (11 EPEC, 11 EAEC, 1 EIEC, and 1 ETEC) were detected as single or mixed infection in 21 specimens by Seegene. By in-house PCR, 23 diarrheagenic *E. coli* (12 EPEC, 8 EAEC, 2 STEC, and 1 EIEC) were detected as single or mixed infection in 19 specimens. The overall percentage agreement between the Seegene and in-house PCR in diarrheagenic *E. coli* was 95% (187 of 197; 95% CI, 0.855–0.961) and 0.849 [0.783–0.915]) with equal PPA (88%) and NPA (100% and 98%). Two *Shigella* spp and 1 *Cryptosporidium* spp were detected by 2 or more assays and thus were regarded as consensus positive.
EPEC and 1 EAEC detected only by in-house PCR). For these 10 specimens, FilmArray was performed; 2 cases showed agreement between Seegene and FilmArray (1 EPEC and 1 EAEC), and the remaining 8 cases showed consistent results between in-house PCR and FilmArray (3 EPEC, 1 EAEC, and 4 negative).

By Luminex, 7 diarrheagenic E. coli (6 O157 and 1 ETEC) were detected as single or mixed infection in 7 specimens. Although the ETEC was also detected by another method (Seegene), of the 6 specimens that showed positive results for E. coli O157, only 1 was also identified as STEC in other assays. The MFI of the true-positive case was 1042, but those of false-positive cases were much lower (157, 158, 201, 303, and 421). By BD MAX, 1 STEC was detected, and it was detected by other methods (in-house PCR and Luminex) (Supplemental Table 1).

**Detection of Multiple Pathogens**

A total of 61 multiple cases were detected. The Seegene, Luminex, and BD MAX assays showed overall rates of mixed infections of 2.9% (16 of 554), 9.2% (51 of 554), and 0.3% (1 of 379), respectively. Enteropathogenic E. coli (6 cases), Campylobacter (6), C. difficile (5), and Salmonella (5) were the common pathogens in mixed infection for Seegene, and Salmonella (29), Campylobacter (27), C. difficile (15), and ETEC (4) were those for Luminex. The most common combination was Campylobacter + Salmonella (19 of 61). In the case of BD MAX, 1 Shigella and 1 EIEC were detected as multiple pathogens. Among the 61 cases of mixed infection, only 3 cases (2 cases of Campylobacter + C. difficile and 1 case of Campylobacter + ETEC) showed concordant results between at least 2 assays.

**Method Concordance Between Seegene Allplex GI and Luminex xTAG GPP for Virus Detection**

The overall agreement of Seegene and Luminex was 0.947 and 0.927, respectively. The PPA of Seegene and Luminex was 99% and 93%, respectively, and NPA was 96% and 99% (Table 2). The x/PPA/NPA of Seegene and Luminex was 0.946/100/97 and 0.967/97/99 for norovirus and 0.974/99/99 and 0.929/100/98 for rotavirus. The relatively lower PPA of Luminex was due to frequent false-negative results (8 cases among the 79 positive cases) in rotavirus detection and 2 false-negative results in norovirus.

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**Table 1. Summary of Targets of Seegene Allplex GI, Luminex xTAG Gastrointestinal Pathogen Panel (GPP), and BD MAX Enteric Panel Assays**

| Targets (N = 24) | Seegene Allplex GI (Seegene, Seoul, Korea) | Luminex xTAG GPP (Luminex Corporation, Austin, Texas) | BD MAX Enteric Panel Assays (BD Diagnostics, Sparks, Maryland) |
|-----------------|------------------------------------------|-----------------------------------------------------|---------------------------------------------------------------|
| **Bacteria**    |                                          |                                                     |                                                               |
| (n = 13)        |                                          |                                                     |                                                               |
| Aeromonas spp   | A                                       | NA                                                  | NA                                                           |
| Campylobacter spp | A                                         | A                                                   | A                                                            |
| Clostridium difficile | A                                    | A                                                   | A                                                            |
| Salmonella spp | A                                        | A                                                   | A                                                            |
| Shigella spp    | A                                        | A                                                   | A                                                            |
| Vibrio cholerae | A                                        | A                                                   | A                                                            |
| Yersinia enterocolitica | A                                         | A                                                   | A                                                            |
| EAEC (aggR)     | A                                       | NA                                                  | NA                                                           |
| EPEC (eaeA)     | A                                       | NA                                                  | NA                                                           |
| Escherichia coli O157 | A                          | A                                                   | A                                                            |
| ETEC (h/s)      | A                                       | A                                                   | A                                                            |
| STEC (stx1/2)   | A                                       | A                                                   | A                                                            |
| EIEC (ipaH)     | A                                       | NA                                                  | A                                                            |
| **Viruses** (n = 5) |                                         |                                                     |                                                               |
| Adenovirus 40/41 | ...                                    | A                                                   | ...                                                          |
| Norovirus GI/GII | ...                                    | A                                                   | ...                                                          |
| Rotavirus A     | ...                                    | A                                                   | ...                                                          |
| Astrovirus      | ...                                    | A                                                   | ...                                                          |
| Sapovirus       | ...                                    | A                                                   | ...                                                          |
| **Parasites** (n = 6) |                                         |                                                     |                                                               |
| Cryptosporidium spp | ...                                | A                                                   | ...                                                          |
| Entamoeba histolytica | ...                              | A                                                   | ...                                                          |
| Giardia lamblia | ...                                   | A                                                   | ...                                                          |
| Blastocystis hominis | ...                             | A                                                   | ...                                                          |
| Dientamoeba fragilis | ...                              | NA                                                  | ...                                                          |
| Cyclospora cayetanensis | ...                               | NA                                                  | ...                                                          |

Abbreviations: A, available; EAEC, enteroaggregative E. coli; EIEC, enteroinvasive E. coli; EPEC, enteropathogenic E. coli; ETEC, enterotoxigenic E. coli; NA, not available; STEC, Shiga toxin 2–producing E. coli; …, not applicable.

a. Bacteria panel 1 of Seegene Allplex GI.
b. Bacteria panel 2 of Seegene Allplex GI.
c. Target for C. hominis and C. parvum only.
Method Concordance Between Stool Culture and Multiplex Assays

Of the 182 stool culture samples, 53 samples were culture positive (34 Campylobacter spp, 17 Salmonella spp, 1 Aeromonas hydrophila, and 1 Vibrio parahaemolyticus). Of the 34 Campylobacter spp–positive samples, Seegene detected 23 cases (67.7%), Luminex 28 (82.4%), and BD MAX 21 (61.8%). For Salmonella spp, of the 18 culture-positive specimens, all 3 multiplex assays detected 14 cases (77.8%). On the contrary, in 4 culture-negative cases, Campylobacter spp was detected both by Seegene and Luminex.

DISCUSSION

To our knowledge, this is the first study comparing Seegene, Luminex, and BD MAX for detecting GI pathogens. Forty GI pathogens (15 Campylobacter spp, 18 C difficile, 3 Salmonella spp, 2 Shigella spp, 1 Cryptosporidium spp, and 1 adenovirus) were additionally identified compared with routine methods. This finding emphasizes that the most important value of these molecular tests is the capability of detecting GI pathogens that would otherwise be overlooked in routine diagnostic procedures, which is in line with a previous study that reported a higher detection rate for GI pathogens using FilmArray (33.0%) and Luminex assay (30.3%) than conventional assay (8.3%). It is noteworthy that all but 2 of the 40 additionally identified GI pathogens were identified in specimens submitted for multiplex PCR for diarrheagenic E coli and Salmonella spp or routine bacterial culture. Among the 175 specimens submitted for C difficile assay, only 1 Salmonella spp was additionally identified. This finding indicates that these multiplex assays are more useful for patients with community-onset diarrhea rather than for patients with suspected antibiotic-associated diarrhea. Interestingly, 1 Cryptosporidium spp, which is one of the most common protozoan infections and frequently detected in traveler’s diarrhea in children, was additionally identified. Among the 304 specimens submitted for norovirus/rotavirus examinations, 1 adenovirus was additionally detected. Enteric adenovirus infection is a common cause of infantile diarrhea, though less common than rotavirus infection. In our study, the adenovirus-positive sample was from an infant having diarrhea. Adenovirus is one of the most significant etiologic agents of childhood viral gastroenteritis in industrialized countries, and adenoviruses 40 and 41 are among the most common etiologic agents of acute gastroenteritis among infants and children younger than 2 years.

According to the assay format, Seegene, Luminex, and BD MAX additionally identified 39, 40, and 12 pathogens, respectively. The small number of additionally identified GI pathogens with BD MAX is mainly because the BD MAX enteric panel does not contain a target for C difficile and due to the lower PPA of BD MAX (79%) for Campylobacter, Salmonella, and Shigella than those of Seegene (88%) and Luminex (93%). Although many evaluations of these multiplex panels have demonstrated that C difficile is one of the most common organisms detected, whether detection of C difficile represents true infection or colonization will be an important area for future research.

In general, the 3 multiplex kits showed almost perfect agreement (κ > 0.8) except for Luminex against Salmonella spp (κ = 0.454). The low κ value of Luminex was due to frequent false positives in Salmonella (n = 31). This is in line with a Vietnamese study that compared the Luminex assay with conventional culture and/or real-time PCR; the sensitivity and specificity of Luminex GPP assay were more than 88% for all compared pathogens (3 bacteria and 4 viruses), except for Salmonella, for which the specificity was very low (66.8%). In contrast, other several studies showed high specificity for Salmonella in Luminex. In contrast to the previous report in which a real-time PCR showed superior sensitivity to bacterial culture in detecting Salmonella spp and Campylobacter spp, in our study, the sensitivity of the 3 molecular methods for detecting culture-positive Salmonella spp was identical at 77.8%. This might be due to the use of enrichment broth in our laboratory and/or degradation of nucleic acids during the storage of raw stool.

Table 2. Results of Bacteria, Parasite, and Virus by Comparative Evaluation Among Seegene Allplex GI, Luminex xTAG Gastrointestinal Pathogen Panel, and BD MAX Enteric Assays

| Organism                  | Seegene | Luminex | BD MAX |
|---------------------------|---------|---------|--------|
|                           | PPA     | NPA     | TP     | FN  | PPA | NPA | TP | FN  | PPA | NPA | TP | FN  |
| Campylobacter spp (38)    | 0.932   | 88      | 100    | 37  | 0   | 337 | 5  | 0.905 | 100 | 98  | 42  | 10  | 327 | 0  | 0.831 | 76  | 100 | 32  | 2   | 335 | 10 |
| Shigella spp (3)          | 0.908   | 88      | 100    | 66  | 1   | 478 | 9  | 0.849 | 88  | 98  | 64  | 13  | 466 | 11 | NA     | NA   | NA  | NA  | NA  | NA  | NA |
| Cryptosporidium spp (1)   | 0.946   | 100     | 97     | 63  | 5   | 236 | 0  | 0.967 | 97  | 99  | 61  | 1   | 240 | 2  | NA     | NA   | NA  | NA  | NA  | NA  | NA |
| Rotavirus (79)            | 0.974   | 99      | 99     | 78  | 2   | 223 | 1  | 0.929 | 90  | 100 | 71  | 1   | 224 | 8  | NA     | NA   | NA  | NA  | NA  | NA  | NA |
| Adenovirus (1)            | 0.900   | 100     | 100    | 1   | 0   | 303 | 0  | 0.900 | 100 | 100 | 1   | 0   | 303 | 0  | NA     | NA   | NA  | NA  | NA  | NA  | NA |
| N: True-Positive          | 100     | 100     | 100    | 100 | 100 | 100 | 100 | 100  | 100 | 100 | 100 | 100 | 100 | 100 | 100  | 100 | 100 | 100 | 100 | 100 |

Abbreviations: FN, false-negative; FP, false-positive; NA, not available; NPA, negative percentage agreement; PPA, positive percentage agreement; TN, true-negative; TP, true-positive.

a Targeted by Seegene and Luminex, but not by BD.
b As the BD MAX Enteric bacterial panel does not contain target for C difficile, specimens positive for C difficile by either Seegene or Luminex were confirmed by Xpert C difficile assay.
c As virus detection kit is not available for BD MAX, the results of Seegene and Luminex assays were compared with antigen test.
but in 4 culture-negative cases, they were additionally detected by both Seegene and Luminex. In general, PCR assay has a sensitivity and specificity for Campylobacter and Salmonella equivalent to that of stool culture.\textsuperscript{5,11} However, in the case of Salmonella spp, there are some contradicting reports: Although Harrington et al\textsuperscript{23} reported that the BD MAX enteric bacterial panel demonstrated superior sensitivity and reliably detected Salmonella, enterohemorrhagic E coli (EHEC) O157, Shigella, and Campylobacter at concentrations 10\textsuperscript{-1} to 10\textsuperscript{-2} lower than those needed for stool culture, Cunningham et al\textsuperscript{24} reported greater sensitivity of routine stool culture for Salmonella spp because of enrichment in selenite broth. Onori et al\textsuperscript{1} also demonstrated greater sensitivity for multiplex PCR than our routine methods, with the exception of Salmonella spp and toxigenic C difficile detection.

The data for clinical significance of mixed infections are still insufficient, and laboratorians and clinicians find it difficult to interpret the results. More studies are needed to solve many issues related to coinfections, including the clinical impact of multiple pathogens on disease severity and management. Based on our data, the percentage of mixed infection was the highest in Luminex (9.2%), followed by BD MAX (0.3%). The high rate of mixed infection in Luminex is in line with a previous report\textsuperscript{13} in which Luminex showed a mixed-infection rate of 14.1%. In our study, the Campylobacter spp and Salmonella spp combination was the most common mixed-pathogen combination detected by Luminex. This result might be due to the many false positives of Salmonella spp in Luminex. Among the 19 Campylobacter spp and Salmonella spp combinations, only 1 case was true-positive for Salmonella spp. The specimens that gave false-positive results had much lower MFI of probe 1 compared with those of true-positive cases (430 versus 1394; \textit{P} < .001). The Luminex xTAG GPP assay applies only 1 cutoff value for each target. However, for Salmonella, it uses 2 kinds of cutoff values: if probe 1 signal is above 1400, it means positive; however, if probe 1 signal is between 200 and 1400, probe 2 must also be above 200 for the result to be positive. Given that the limit of detection of Luminex for Salmonella spp is higher (1.56 \times 10^8 CFU/mL) than that of Seegene (2.5 \times 10^8 CFU/mL) or BD MAX (4.4 \times 10^8 CFU/mL), the possibility of cross-reactivity with an alternative component of the GI microbiota present in this population cannot be excluded. In addition, we cannot rule out the possibility of amplicon contamination because, the Luminex platform is an open system and the operator must handle the PCR product before hybridization.

Diarrheagenic E coli is recognized as a significant cause of epidemic and endemic diarrhea worldwide.\textsuperscript{25} It can be divided into 5 main pathotypes: EAEc, EHEC/STEC (including O157), EIEC, EPEC, and ETEC. They are the main causative agents for infectious enterocolitis, and they have different virulence factors that help them to cause diseases by different mechanisms, resulting in variable clinical symptoms.\textsuperscript{26} In this study, EPEC was the most common pathogen (60%; 9 of 15), followed by EAEc (40%; 6 of 15), STEC (7%; 1 of 15), EIEC (7%; 1 of 15), and ETEC (7%; 1 of 15). In this comparative study, the Seegene assay and in-house PCR showed substantial agreement (\textit{k} = 0.722). However, for 10 discrepant cases, in-house PCR showed higher agreement (8 of 10) with FilmArray than Seegene (2 of 10). Luminex showed a high false-positive rate (5 of 6) in identifying E coli O157. Similar to the cases with Salmonella spp, 5 false-positive specimens showed much lower MFI (248) than true-positive specimens (1042).

Luminex showed 2 false-negative results for norovirus and 8 for rotavirus. This result is in line with previous studies\textsuperscript{27,28} showing that Luminex had lower sensitivity in norovirus and rotavirus compared with FilmArray and TaqMan Array Card. Zhuo et al\textsuperscript{29} reported that Luminex had lower sensitivity especially in detecting norovirus genotype II, and the 2 false-negative cases in our study were norovirus genotype II by Seegene.

Regarding the practical application of the assays, Seegene Allplex GI is a multiplex 1-step reverse transcription real-time PCR assay that is composed of 4 panels (bacteria I, bacteria II, virus, and parasite) that can be selected according to the various patient conditions, for example, virus and bacteria I panels for hospitalized patients with suspected nosocomial infection or bacteria I and II panels for patients with occult blood in stool. Seegene Allplex GI showed the highest overall PPA (94%; 258 of 275) and NPA (98%; 571 of 583), respectively.

Luminex xTAG GPP is a multiplex molecular-based approach that can simultaneously detect its 15 targets.\textsuperscript{5,20} It can process 96 samples in a single run, but requires 5 hours for analysis and a high level of technical skill to prevent cross-contamination because the operator must handle the PCR product before the hybridization step. The BD MAX system is a walkaway PCR instrument that can process a specimen by amplifying and detecting the nucleic acids in a batch of up to 24 samples in up to 3 hours using only 1.25 minutes of hands-on time per sample.\textsuperscript{21} In bacterial pathogens except C difficile, the BD MAX Enteric assay showed second place in \textit{k} agreement, with overall percentage agreement of 96% (362 of 379). However, it was less sensitive than Seegene or Luminex for detecting Campylobacter spp, showing a high false-negative rate (24%; 10 of 42), although it showed superior sensitivity to the culture method in other studies.\textsuperscript{22,29}

This study has several limitations. First, as the targets included in each assay are different, we had to compare them with other assays, such as Xpert for C difficile and EIA for norovirus/rotavirus. Second, we could not perform discrepancy analysis because of study complexity. Another limitation is that routine bacterial culture was tested in only some samples because of the variety of sample sources (samples submitted for either culture, PCR or C difficile test, or virus antigen tests).

In conclusion, the 3 multiplex molecular assays showed substantial to almost perfect agreement, and they allowed additional identification of GI pathogens. These multiplex molecular assays appear to be a promising tool for the simultaneous detection of multiple GI pathogens. To decide which of these assays to use as a routine diagnostic procedure, various factors, such as range of pathogens detected, cost, throughput, hands-on time, and required technical skills, should be considered.

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