Use and Interpretation of Enteropathogen Multiplex Nucleic Acid Amplification Tests in Patients With Suspected Infectious Diarrhea

Harika Yalamanchili, DO, Dima Dandachi, MD, and Pablo C. Okhuysen, MD

Dr Yalamanchili recently completed a fellowship in infectious diseases at The University of Texas Health Science Center at Houston/UT MD Anderson Cancer Center in Houston, Texas. Dr Dandachi is an assistant professor of medicine at the University of Missouri Health Care in Columbia, Missouri. Dr Okhuysen is a professor of medicine in the Department of Infectious Diseases, Infection Control, and Employee Health at The University of Texas MD Anderson Cancer Center.

Address correspondence to:
Dr Pablo C. Okhuysen
Department of Infectious Diseases, Infection Control, and Employee Health
The University of Texas MD Anderson Cancer Center
1515 Holcombe Blvd, Unit 1460
Houston, TX 77030
Tel: 713-745-8413
Fax: 713-745-6839
E-mail: pcokhuysen@mdanderson.org

Keywords
Infectious diarrhea, multiplex polymerase chain reaction, nucleic acid amplification tests, enterotoxigenic *E coli*, enteropathogenic *E coli*, enteroaggregative *E coli*

Abstract: Acute diarrheal illness due to gastrointestinal infection is a significant cause of morbidity and mortality in the United States and around the world. Determining the causative organism in a timely manner assists with patient care, identifying outbreaks, providing infection control, and administering antimicrobial therapy when indicated. Traditional diagnostic modalities based on culture and immunoassays are limited by their low sensitivity and long turnaround time. Nucleic acid amplification tests (NAATs) for enteric pathogens allow for the syndromic testing of stool for multiple pathogens simultaneously and have higher sensitivity with a shorter turnaround time. However, by not isolating the organism, NAATs do not provide drug susceptibility or confirmatory identification. Furthermore, NAATs cannot distinguish between true infection and carrier states. Nevertheless, several studies have demonstrated the cost-effectiveness of multiplex NAATs by reducing the length of hospital stay and cost of isolation. Five platforms are currently approved by the US Food and Drug Administration that can detect different bacteria, parasites, and viruses. The sensitivity and specificity of each platform depends on the targeted pathogens and whether the tests are performed on fresh stool, frozen stool, or in transport media. Overall, these tests have high sensitivity and specificity of more than 90% when used in symptomatic patients. Thus, multiplex NAAT gastrointestinal platforms offer several advantages compared to traditional methods. However, the interpretation of the results requires acknowledging the limitations of the tests and exercising clinical judgment. More studies are needed to establish the cost-effectiveness of multiplex NAATs and their impact on antibiotic stewardship and clinical outcomes.

Acute diarrheal illness due to gastrointestinal (GI) infection is a leading cause of outpatient visits and hospitalizations among US residents and travelers.[1] Most episodes are self-limited events. For elderly patients, immunosuppressed patients, and patients with prolonged or severe disease, determining the specific

microbial etiology is important to provide antimicrobial therapy when indicated and avoid inappropriate antibiotics. In addition, rapid and accurate detection of enteropathogens can expedite their identification and contain food or waterborne outbreaks.[2]

Prior to the advent of multiplex nucleic acid amplification tests (NAATs), the comprehensive evaluation of patients with suspected GI infection required performing bacterial cultures using a series of selective culture media, stains for ova and parasites, enzyme immunoassays, and single-agent polymerase chain reaction (PCR)-based NAATs. These conventional clinical microbiology laboratory techniques, which are still the standard at many centers, frequently fail to reveal a causative agent due to low sensitivity and use of prior antibiotic therapy, and are plagued by long turnaround times and excessive costs due to the labor involved. In addition, this piecemeal clinical evaluation has low sensitivity because it depends on health care providers ordering specific tests for suspected organisms, frequently missing others given the significant overlap in clinical presentations. An example is norovirus, a common cause of gastroenteritis, which can, in adults, present only with watery diarrhea without nausea or emesis.[3] The shift in clinical microbiology toward the implementation of multiplex NAATs has significant implications for physicians, patients, and public health in general.[4] In this article, we discuss the use, interpretation, and limitations of GI multiplex NAATs in the diagnosis of suspected infectious diarrhea in adults. We also review the characteristics of the US Food and Drug Administration (FDA)-approved platforms and the features needed in the next generation of GI enteropathogen multiplex NAATs.

Rationale for Using Multiplex Nucleic Acid Amplification Tests for the Diagnosis of Gastrointestinal Infections

Multiplex NAATs have a high sensitivity for the detection of enteropathogens. This was exemplified in a large multicenter European study that compared the use of the BioFire FilmArray Gastrointestinal Panel (BioFire Diagnostics) against traditional laboratory methods. The panel detected at least 1 organism in 54% of the samples, whereas the local laboratory protocols detected at least 1 organism in only 18% of the samples.[5] Multiplex NAAT results are available within hours and, when negative, can greatly affect infection control practices in hospital settings.[6] The various GI multiplex NAATs approved for use in the US market test for an array of enteropathogens simultaneously (Table 1), including common viruses such as norovirus and rotavirus, along with intestinal bacteria, including *Clostridium difficile* and Shiga toxin–producing *Escherichia coli*. In some instances, multiplex NAATs can also identify other diarrheagenic *E coli* (DEC) pathotypes, such as enterotoxigenic *E coli* (ETEC), enteropathogenic *E coli* (EPEC), and enteroaggregative *E coli* (EAEC). Although the significance of identifying EPEC and EAEC in diarrheal stools of US adults is still up for debate, a recent study demonstrated that both pathotypes are commonly identified in patients with cancer and in immunosuppressed patients with diarrhea acquired in the United States.[7] Multiplex NAAT platforms also vary in the number of specimens that can be tested simultaneously and in turnaround time (Table 2).

Although a detailed clinical and exposure history and physical examination can be useful in identifying risk factors for bacterial, protozoal, or viral causes of diarrhea, and traditionally have been used to guide the workup of stool samples, there is significant overlap in the clinical presentations of many of the causative agents. Due to the poor predictive value of targeted testing, a syndromic approach that considers a larger number of pathogens may be preferred. This type of approach has been the standard of care for many years in the diagnosis of respiratory illnesses. In an era of increasing comorbidities, international travel, and use of immunosuppressants, a syndromic approach for GI infections allows for a higher probability of making a timely, accurate, and cost-effective diagnosis.

Limitations of Using Multiplex Nucleic Acid Amplification Tests for the Diagnosis of Gastrointestinal Infections

The high sensitivity of GI multiplex NAATs and a syndromic catch-all approach can potentially lead to finding mixed infections that are difficult to interpret or the identification of carriers excreting a low number of enteropathogens. For example, more than 1 pathogen was identified in 13.0% to 31.5% of patients with diarrhea in various reports[5,8,9] and in up to 69.0% of controls in a case-control study conducted in the Ivory Coast.[10]

The Centers for Disease Control and Prevention has considered culture-independent diagnostic tests for detection of enteric pathogens to be a threat to public health surveillance.[4] Current methods of enteric disease surveillance are based mostly on culture-confirmed infections. Isolates are still needed for antimicrobial susceptibility testing, serotyping, subtyping, pulse-typing, and whole-genome sequencing to monitor trends in clonal spread and resistance; to identify and investigate outbreaks in a timely manner; and to remove contaminated products from the market. When stool specimens are collected for molecular testing, they may, in some cases, be incompatible with culture because of the collection methods or media used, such as dry fecal swabs. For *Salmonella*, the

Table 1. Pathogens Detected by Gastrointestinal Multiplex Platforms Approved by the FDA in the United States

	Bacteria	Parasites	Viruses
BioFire FilmArray Gastrointestinal Panel	• *Campylobacter* (*C coli, C jejuni, C upsaliensis*) • *Salmonella* • *Shigella* • Enteroaggregative *Escherichia coli* • Enteropathogenic *Escherichia coli* • Enterotoxigenic *Escherichia coli* heat-labile/heat-stable enterotoxin • Shiga toxin–producing *Escherichia coli* (Shiga toxin 1 and 2) • *Escherichia coli* O157 serogroup • Enteroinvasive *Escherichia coli* • *Plesiomonas shigelloides* • *Vibrio* (*V vulnificus*, *V parahaemolyticus*, *V cholerae*) • *Yersinia enterocolitica* • *Clostridium difficile* (toxin A/B)	• *Cryptosporidium* • *Entamoeba histolytica* • *Giardia lamblia* • *Cyclospora cayetanensis*	• Norovirus GI/GII • Rotavirus A • Adenovirus F40/F41 • Astrovirus • Sapovirus (I, II, IV, V)
BD Max System[a]	• Enterotoxigenic *Escherichia coli* heat-labile/heat-stable enterotoxin • *Plesiomonas shigelloides* • *Vibrio (V vulnificus, V parahaemolyticus, V cholerae)* • *Yersinia enterocolitica* • *Salmonella* • *Shigella* • *Campylobacter* • Shiga toxin–producing *Escherichia coli* (Shiga toxin 1 and 2)	• *Giardia lamblia* • *Cryptosporidium* • *Entamoeba histolytica*	None[b]
Great Basin Stool Bacterial Pathogens Panel	• *Campylobacter (C coli, C jejuni)* • *Salmonella* • *Shigella* • Shiga toxin–producing *Escherichia coli* (Shiga toxin 1 and 2) • *Escherichia coli* O157 serogroup	None	None
Luminex xTAG Gastrointestinal Pathogen Panel	• *Campylobacter* • *Salmonella* • *Shigella* • Enterotoxigenic *Escherichia coli* heat-labile/heat-stable enterotoxin • Shiga toxin–producing *Escherichia coli* (Shiga toxin 1 and 2) • *Escherichia coli* O157 serogroup • *Vibrio cholerae* (cholera toxin gene) • *Clostridium difficile* (toxin A/B)	• *Cryptosporidium* • *Entamoeba histolytica* • *Giardia lamblia*	• Norovirus GI/GII • Rotavirus A • Adenovirus F40/F41
Verigene Enteric Pathogens Nucleic Acid Test	• *Campylobacter (C coli, C jejuni, C lari)* • *Salmonella* • *Shigella (S dysenteriae, S boydii, S sonnei, S flexneri)* • Shiga toxin–producing *Escherichia coli* (Shiga toxin 1 and 2) • *Vibrio* (*V cholerae, V parahaemolyticus*) • *Yersinia enterocolitica*	None	• Norovirus GI/GII • Rotavirus A

FDA, US Food and Drug Administration.

[a]This system consists of the BD Max Extended Enteric Bacterial Panel, the BD Max Enteric Bacterial Panel, and the BD Max Enteric Parasite Panel. Each panel detects a different set of bacteria or parasites. [b]A viral panel is under FDA review.

inability to distinguish serotypes limits the detection and investigation of outbreaks. In the case of Shiga toxin–producing *E coli*, the identification of serogroups requires an isolate derived from culture. Some GI multiplex NAATs are unable to distinguish Shiga toxin–producing *E coli* O:157 from non-O:157 serotypes. Thus, the shift from culture-based methods to diagnostic tests that do not provide isolates impedes the interpretation of public

Table 2. Comparison of Gastrointestinal Multiplex Platforms Approved by the FDA

	Technology	Transport Media	Interference	Detection Time	Throughput
BioFire FilmArray Gastrointestinal Panel	Nested PCR plus melting curve	Stool sample preserved in Cary-Blair media	Low frequency cross-reactivity with commensal organisms	1-2 hours	1 sample assayed on an individual process time
BD Max System[a,b]	Fluorogenic gene-specific hybridization probes for the detection of the amplified DNA	Stool sample preserved in Cary-Blair media or directly on unpreserved stool specimen	Potential interference with topical nystatin cream, spermicidal lubricant, hydrocortisone cream, and Vagisil cream	3-4 hours	1-24 samples in a single batch, can mix and match different assays during the same run
Great Basin Stool Bacterial Pathogens Panel	Colorimetric target-specific hybridization to probe on a chip surface, optical reader, automated software with built-in result interpretation	Stool sample preserved in Cary-Blair media or C&S preservation and transport media	No significant interference	<2 hours	1 sample assayed on an individual process time
Luminex xTAG Gastrointestinal Pathogen Panel	Reverse transcription PCR using proprietary universal sorting system (fluorescent bead–based detection)	Stool sample preserved in Cary-Blair media or raw stool sample	No significant interference	5 hours	Accommodates up to 24 stool samples in a single batch
Verigene Enteric Pathogens Nucleic Acid Test	Target detection based on silver nanoparticles amplifying the signal	Stool sample preserved in Cary-Blair media	No significant interference	2 hours	1 sample assayed on an individual process time

C&S, culture and sensitivity; FDA, US Food and Drug Administration; PCR, polymerase chain reaction.

[a]This system consists of the BD Max Extended Enteric Bacterial Panel, the BD Max Enteric Bacterial Panel, and the BD Max Enteric Parasite Panel. The panels use the same technology and transport media, but the assays detect different pathogens. [b]A viral panel is under FDA review.

health surveillance data and the assessment of prevention effort success.[11] In response, the Association of Public Health Laboratories recommends that, when a pathogen that requires public health notification is detected, reflex culturing of specimens should be performed for bacterial pathogens to obtain isolates.[12] If a clinical laboratory is unable to culture the isolates for public health surveillance purposes, the patient sample should be submitted to local or state public health laboratories by using acceptable specimen types. This additional confirmatory testing adds cost to the clinical laboratory. Although specimen submission is more convenient for clinical laboratories, it will shift the burden and cost to public health laboratories.[4] One way to address this problem is to perform directed cultures that would involve setting up cultures in selective media for the organism identified, rather than with the entire set of conventional differential culture media.

In addition, the clinical significance of an identified organism may be unclear. Multiplex NAATs detect DNA or RNA, which might not indicate infection with a viable organism. They can also detect microorganisms at nonpathogenic levels. This can be problematic because patients can be asymptomatic carriers. For example, prolonged fecal shedding that can last for weeks for norovirus, or for months in the case of *Salmonella*, is well documented.[13,14]

Another concern with the use of GI multiplex NAATs is the possibility of contamination. Contamination may be caused by the operator (more of an issue with respiratory and central nervous system NAAT panels), during the test from carryover contamination from prior samples, or from nearby specimens (especially in laboratories that do not have a designated area for molecular testing).

Beal and colleagues estimated that the overall health care cost was lower when GI multiplex NAATs were used over conventional methods, with the savings mostly attributed to decreases in hospital length of stay.[15]

Among hospitalized patients, additional cost savings can be realized through the reduction of the cost of isolation practices; however, this shifts some of the cost to the clinical laboratory. Goldenberg and colleagues demonstrated that, although an initial investment has to be made to employ GI multiplex panel testing, it significantly reduces the cost by decreasing the time patients remain in contact isolation.[16]

Because the use of these platforms requires that samples be placed in transport media, the technicians processing the specimen are not able to determine if the stool is formed or not prior to testing. This can lead to inappropriate testing, identification of colonized patients, and, potentially, unnecessary treatment. Proper education must be enforced, ideally starting at the nursing level to collect and process only unformed stool samples.

Another limitation is the detection of pathogens that have not been clearly associated with diarrheal disease, as is the case of EPEC and EAEC in US adults.[7] Additionally, test of cure using a GI multiplex panel is not considered appropriate because over 50% of patients can remain positive for the same target 4 weeks after initial testing.[17]

Gastrointestinal Multiplex Nucleic Acid Amplification Test Platforms Approved by the US Food and Drug Administration

There are currently 5 FDA-approved GI multiplex NAAT platforms in the United States. These panels have similar sensitivity and specificity but detect different sets of bacteria, viruses, parasites, or combinations of these organisms.[18] All of the available GI multiplex NAATs are strictly qualitative.

The Luminex xTAG Gastrointestinal Pathogen Panel (GPP; Luminex Corporation) was the first FDA-approved multiplex test for the detection and identification of multiple GI pathogens and was the first FDA-cleared device for nucleic acid–based testing of norovirus, obtaining clearance in 2013. This panel is intended for the simultaneous qualitative detection and identification of 14 viral, parasitic, and bacterial nucleic acids with a 5-hour turnaround. For GPP testing, fresh or frozen-thawed stool is placed in Cary-Blair transport media. The stool specimen requires pretreatment followed by nucleic acid extraction, multiplex PCR, bead hybridization, and, finally, data analysis. It is an open system, so there is a theoretical risk of contamination; thus, good laboratory practices are important. In a multicenter study of pediatric and adult patients conducted in Canada using the 15-target, Canadian-approved GPP assay, which includes *Yersinia enterocolitica* detection, the sensitivity for 12 of the 15 targets was 94.3%, and the specificity across all 15 targets was 98.5%.[19] There have been reports of reduced sensitivity for *Salmonella*.[20] The GPP was used as a rapid screening diagnostic method during the 2011 outbreak of enterohemorrhagic *E coli* in Germany.[21] Additionally, it was used in the 2010 cholera outbreak in Haiti in parallel with culture for *Vibrio cholerae* and *Salmonella* for confirmed cases.[22]

The BioFire FilmArray Gastrointestinal Panel was approved by the FDA in 2014 and is a system that allows for detection of 22 enteropathogens. The sample is placed in Cary-Blair transport media. The user then injects hydration solution, and the sample is combined with sample buffer mix in the provided pouch. The panel extracts and purifies all nucleic acids from the sample, and then performs a nested multiplex amplification. The panel includes assays for different bacteria, focusing on the targets of the most commonly implicated organisms in gastroenteritis. The panel contains a single assay for *Vibrio* spp that can detect *V parahaemolyticus, V vulnificus*, and *V cholerae* and may also be able to detect other, less common species, including *V alginolyticus, V fluvialis*, and *V mimicus*. The panel can detect 6 pathotypes of DEC. However, because the horizontal transfer of genes between organisms has been documented, positive results for multiple DEC can occur. In addition, the platform cannot distinguish between infection due to enteroinvasive *E coli* and infection with *Shigella* because the probe target is shared by both organisms. It has been noted that there is some component of low frequency cross-reactivity between certain pathogens and commensal microorganisms, such as in the case of *Giardia lamblia* with *Bifidobacterium* and *Ruminococcus*. Of interest, the panel detects both adenovirus F40 and F41, which will not cross-react with respiratory adenoviruses. In a multicenter prospective study by Buss and colleagues, this panel was compared with conventional stool culture and molecular methods in 1556 specimens.[8] The study demonstrated a sensitivity or positive percent agreement (PPA) of 100% for 12 of 22 targets and 94.5% for the remaining targets. Of note, it was not possible to assess the sensitivity/PPA of the panel for detecting *Vibrio* spp, including *V cholerae*, or *Entamoeba histolytica*, as these organisms were not detected by the employed comparator methods, or at all in the case of *E histolytica*. The specificity or negative percent agreement (NPA) of the panel was 97.1% for all panel targets.

The BD Max System (Becton, Dickinson and Company) can be used with fresh stool or stool placed in Cary-Blair transport media. The BD Max System consists of 3 FDA-approved assays, which can be performed in the same run. The BD Max Extended Enteric Bacterial Panel detects the DNA of 4 bacteria: *Plesiomonas shigelloides*, *Vibrio* (*V vulnificus, V parahaemolyticus,* and *V cholerae*), ETEC heat-labile enterotoxin/heat-stable enterotoxin genes, and *Y enterocolitica*. In a multicenter study, this

panel showed a PPA and NPA greater than 95% for the detection of these bacteria in stool specimens from symptomatic patients.[23] The BD Max Enteric Bacterial Panel identifies *Salmonella, Campylobacter, Shigella,* and Shiga toxin–producing *E coli*. When compared to conventional stool culture, this panel showed a higher sensitivity and specificity.[24] The BD Max Enteric Parasite Panel detects *Giardia lamblia*, *E histolytica, Cryptosporidium parvum,* and *Cryptosporidium hominis*. The performance of this assay in clinical studies has not been consistent, and the results have been difficult to compare, as many studies had a limited number of positive clinical samples and used either microscopy, which is highly dependent on the operator's skills, or in-house PCR as the reference method. However, overall, this assay can be used as a substitute for microscopic examination or immunoassays, with reduced labor time and complexity.[25-28] Finally, the BD Max Enteric Viral Panel, which is currently under FDA review, detects norovirus, rotavirus, adenovirus F40/F41, sapovirus, and astrovirus.

In 2017, the Great Basin Stool Bacterial Pathogens Panel (Great Basin Scientific) received FDA clearance. The molecular test is performed directly from stool in Cary-Blair or culture-and-sensitivity media. It detects *Campylobacter*, *Salmonella*, *Shigella*, and Shiga toxin–producing *E coli*, including O157 and other Shiga toxin 1– and 2–producing serotypes. In a multicenter clinical study performed in stools transported in modified Cary-Blair media, this multiplex NAAT detected 97.1% of the targeted organisms compared to 50.0% when using conventional cultures and enzyme immunoassays.[29] In a retrospective study using frozen stool samples, the PPA was at least 90% (94%-100%), and the NPA was 100% for all analytes excluding *Campylobacter*.[30]

The Verigene Enteric Pathogens Nucleic Acid Test (Luminex Corporation) was approved by the FDA in 2014. The test detects *Campylobacter, Salmonella, Shigella, Vibrio*, and *Y enterocolitica* in addition to norovirus and rotavirus. It also can identify Shiga toxin genes 1 and 2 separately. The PPA of prospectively collected samples ranged from 71% for rotavirus to 95% for *Shigella*, and the NPA was more than 99%.[31]

Unanswered Questions

Given the increased sensitivity, multiplex NAATs have the potential to redefine the epidemiology, disease burden, and natural history of enteric infections; however, additional detailed epidemiologic and case-controlled studies are needed. Other areas in need of clarity include the interpretation of persistently positive GI multiplex NAATs following treatment, particularly in immunosuppressed patients; the importance of co-occurring pathogens; how to best approach patients in whom EPEC and EAEC are identified; and how to interpret samples with discordant results (eg, positive results from a GI multiplex NAAT but negative results from culture). The development of real-time quantitative assays may aid in the interpretation of results and in addressing some of these issues. In the longer term, culture-independent methods that serve clinical diagnostic needs and can provide subtyping information to distinguish strains and drug susceptibility results are needed.[32]

Features of an ideal next-generation multiplex NAAT for GI pathogens should include being small, portable, self-contained, and capable of performing sample preparation, molecular processing, data analysis, and results reporting. This platform should be available at any time of the day and have the capacity for use at the bedside or in the outpatient clinic with individual specimens rather than testing batches of samples. It could be run by individuals with minimal training and should require little or no sample preparation prior to testing and no intervention by the operator between analytic steps. In addition to syndromic-based testing for multiple common enteropathogens, the ideal multiplex NAAT platform for GI pathogens should have high sensitivity and specificity, along with the ability to discern between carriers and patients with an active infection. This can potentially be achieved by performing quantitative measurements of bacterial, parasitic, or viral burden; identifying the expression of virulence factors; characterizing the diversity of the coexisting microbiome; and detecting host biomarkers (such as inflammatory markers) based on well-established and clinically validated cutoff thresholds. Rapid turnaround time could mitigate unnecessary patient isolation, the need for additional testing, and unnecessary therapeutic interventions. Cost efficiencies would be realized owing to shorter duration of hospital stay, decreased utilization of infection prevention interventions, and lower antibiotic usage. None of the currently available FDA-approved GI multiplex NAATs fulfills all of these characteristics.

There is a high cost associated with the initial investment needed to acquire any of the currently available GI multiplex NAATs, including the cost associated with equipment, reagents, training, local validation, and maintenance. Additionally, there are limited data supporting improved patient outcomes or a cost-benefit to providers.

Conclusion

GI multiplex NAATs provide the opportunity to determine potential causes of infectious diarrhea in an efficient and sensitive format. The utility of investing in one of these platforms is based on an individual institution's needs, patient population, financial capacity, and laboratory

capabilities. These platforms represent a wave of new technology that can significantly aid in diagnostic efforts but should always be used in appropriate clinical settings for optimum application. The improved detection of a variety of previously unreported pathogens can sometimes provide more questions than answers, and interpretation requires the use of clinical judgment.

The authors have no relevant conflicts of interest to disclose.

References

1. Riddle MS, DuPont HL, Connor BA. ACG Clinical Guideline: diagnosis, treatment, and prevention of acute diarrheal infections in adults. *Am J Gastroenterol*. 2016;111(5):602-622.
2. Shane AL, Mody RK, Crump JA, et al. 2017 Infectious Diseases Society of America Clinical Practice Guidelines for the diagnosis and management of infectious diarrhea. *Clin Infect Dis*. 2017;65(12):1963-1973.
3. Koo HL, Ajami NJ, Jiang ZD, et al. Noroviruses as a cause of diarrhea in travelers to Guatemala, India, and Mexico. *J Clin Microbiol*. 2010;48(5):1673-1676.
4. Shea S, Kubota KA, Maguire H, et al. Clinical microbiology laboratories' adoption of culture-independent diagnostic tests is a threat to foodborne-disease surveillance in the United States. *J Clin Microbiol*. 2016;55(1):10-19.
5. Spina A, Kerr KG, Cormican M, et al. Spectrum of enteropathogens detected by the FilmArray GI Panel in a multicentre study of community-acquired gastroenteritis. *Clin Microbiol Infect*. 2015;21(8):719-728.
6. Hanson KE, Couturier MR. Multiplexed molecular diagnostics for respiratory, gastrointestinal, and central nervous system infections. *Clin Infect Dis*. 2016;63(10):1361-1367.
7. Chao AW, Bhatti M, DuPont HL, Nataro JP, Carlin LG, Okhuysen PC. Clinical features and molecular epidemiology of diarrheagenic Escherichia coli pathotypes identified by fecal gastrointestinal multiplex nucleic acid amplification in patients with cancer and diarrhea. *Diagn Microbiol Infect Dis*. 2017;89(3):235-240.
8. Buss SN, Leber A, Chapin K, et al. Multicenter evaluation of the BioFire FilmArray Gastrointestinal Panel for etiologic diagnosis of infectious gastroenteritis. *J Clin Microbiol*. 2015;53(3):915-925.
9. Khare R, Espy MJ, Cebelinski E, et al. Comparative evaluation of two commercial multiplex panels for detection of gastrointestinal pathogens by use of clinical stool specimens. *J Clin Microbiol*. 2014;52(10):3667-3673.
10. Becker SL, Chatigre JK, Gohou JP, et al. Combined stool-based multiplex PCR and microscopy for enhanced pathogen detection in patients with persistent diarrhoea and asymptomatic controls from Côte d'Ivoire. *Clin Microbiol Infect*. 2015;21(6):591.e1-591.e10.
11. Huang JY, Henao OL, Griffin PM, et al. Infection with pathogens transmitted commonly through food and the effect of increasing use of culture-independent diagnostic tests on surveillance—Foodborne Diseases Active Surveillance Network, 10 U.S. sites, 2012-2015. *MMWR Morb Mortal Wkly Rep*. 2016;65(14):368-371.
12. Submission of enteric pathogens from positive culture-independent diagnostic test specimens to public health. Interim guidelines. Association of Public Health Laboratories. https://www.aphl.org/aboutAPHL/publications/Documents/FS-Enteric_Pathogens_Guidelines_0216.pdf. Published February 2016. Accessed September 7, 2018.
13. Murata T, Katsushima N, Mizuta K, Muraki Y, Hongo S, Matsuzaki Y. Prolonged norovirus shedding in infants <or=6 months of age with gastroenteritis. *Pediatr Infect Dis J*. 2007;26(1):46-49.
14. Buchwald DS, Blaser MJ. A review of human salmonellosis: II. Duration of excretion following infection with nontyphi Salmonella. *Rev Infect Dis*. 1984;6(3):345-356.
15. Beal SG, Tremblay EE, Toffel S, Velez L, Rand KH. A gastrointestinal PCR panel improves clinical management and lowers health care costs. *J Clin Microbiol*. 2017;56(1):e01457-17.
16. Goldenberg SD, Bacelar M, Brazier P, Bisnauthsing K, Edgeworth JD. A cost benefit analysis of the Luminex xTAG Gastrointestinal Pathogen Panel for detection of infectious gastroenteritis in hospitalised patients. *J Infect*. 2015;70(5):504-511.
17. Park S, Hitchcock MM, Gomez CA, Banaei N. Is follow-up testing with the FilmArray gastrointestinal multiplex PCR panel necessary? *J Clin Microbiol*. 2017;55(4):1154-1161.
18. Nucleic acid based tests. US Food and Drug Administration. https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm. Accessed March 09, 2018.
19. Claas EC, Burnham CA, Mazzulli T, Templeton K, Topin F. Performance of the xTAG® Gastrointestinal Pathogen Panel, a multiplex molecular assay for simultaneous detection of bacterial, viral, and parasitic causes of infectious gastroenteritis. *J Microbiol Biotechnol*. 2013;23(7):1041-1045.
20. Deng J, Luo X, Wang R, et al. A comparison of Luminex xTAG® Gastrointestinal Pathogen Panel (xTAG GPP) and routine tests for the detection of enteropathogens circulating in Southern China. *Diagn Microbiol Infect Dis*. 2015;83(3):325-330.
21. Malecki M, Schildgen V, Kamm M, Mattner F, Schildgen O. Rapid screening method for multiple gastroenteric pathogens also detects novel enterohemorrhagic Escherichia coli O104:H4. *Am J Infect Control*. 2012;40(1):82-83.
22. Valcin CL, Severe K, Riche CT, et al. Predictors of disease severity in patients admitted to a cholera treatment center in urban Haiti. *Am J Trop Med Hyg*. 2013;89(4):625-632.
23. Simner PJ, Oethinger M, Stellrecht KA, et al. Multisite evaluation of the BD Max Extended Enteric Bacterial Panel for detection of Yersinia enterocolitica, enterotoxigenic Escherichia coli, Vibrio, and Plesiomonas shigelloides from stool specimens. *J Clin Microbiol*. 2017;55(11):3258-3266.
24. Harrington SM, Buchan BW, Doern C, et al. Multicenter evaluation of the BD Max Enteric Bacterial Panel PCR assay for rapid detection of Salmonella spp., Shigella spp., Campylobacter spp. (C. jejuni and C. coli), and Shiga toxin 1 and 2 genes. *J Clin Microbiol*. 2015;53(5):1639-1647.
25. Madison-Antenucci S, Relich RF, Doyle L, et al. Multicenter evaluation of BD Max Enteric Parasite real-time PCR assay for detection of Giardia duodenalis, Cryptosporidium hominis, Cryptosporidium parvum, and Entamoeba histolytica. *J Clin Microbiol*. 2016;54(11):2681-2688.
26. Perry MD, Corden SA, Lewis White P. Evaluation of the BD MAX Enteric Parasite Panel for the detection of Cryptosporidium parvum/hominis, Giardia duodenalis and Entamoeba histolytica. *J Med Microbiol*. 2017;66(8):1118-1123.
27. Batra R, Judd E, Eling J, Newsholme W, Goldenberg SD. Molecular detection of common intestinal parasites: a performance evaluation of the BD Max™ Enteric Parasite Panel. *Eur J Clin Microbiol Infect Dis*. 2016;35(11):1753-1757.
28. Mölling P, Nilsson P, Ennefors T, et al. Evaluation of the BD Max Enteric Parasite Panel for clinical diagnostics. *J Clin Microbiol*. 2016;54(2):443-444.
29. Granato PA, Unz MM, Dien Bard J, et al. Comparative evaluation of a PCR amplification and array detection stool bacterial pathogens panel with conventional methods for detecting common bacterial enteric pathogens. Presented at ASM Microbe 2017; June 1-5, 2017; New Orleans, LA. Abstract 1390.
30. Faron ML, Ledeboer NA, Connolly J, et al. Clinical evaluation and cost analysis of Great Basin Shiga toxin direct molecular assay for detection of Shiga toxin-producing Escherichia coli in diarrheal stool specimens. *J Clin Microbiol*. 2017;55(2):519-525.
31. Huang RS, Johnson CL, Pritchard L, Hepler R, Ton TT, Dunn JJ. Performance of the Verigene® Enteric Pathogens Test, Biofire FilmArray™ Gastrointestinal Panel and Luminex xTAG® Gastrointestinal Pathogen Panel for detection of common enteric pathogens. *Diagn Microbiol Infect Dis*. 2016;86(4):336-339.
32. Relman DA. Metagenomics, infectious disease diagnostics, and outbreak investigations: sequence first, ask questions later? *JAMA*. 2013;309(14):1531-1532.