

Stool-Based Diagnostic Testing for Bacterial and Parasitic Gastrointestinal Infections

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Abstract: There have been substantial advancements in microbial stool testing over the past decade, with an increasing range of potential modes of testing. Rapid multiplex molecular panels are powerful tools that can screen for more than 20 pathogens, give results in only a few hours, and have a sensitivity of 95% or higher. However, these tests have their limitations. They are unable to provide antimicrobial susceptibility data, often detect nucleic acid from dead organisms, and can frequently flag codetections with unclear clinical significance. Stool culture, an older modality that requires more time, is the only mode of stool testing that provides information on antibiotic susceptibility needed for targeted therapy and that is optimal for outbreak tracking used by local health departments. These advancements in modes of diagnostic testing have complicated the initial decision of selecting the right test for various clinical contexts. There are now a plethora of stool-based diagnostic options available for common bacterial enteropathogens, protozoal and parasitic organisms, and for the less common organisms encountered in immunocompromised hosts. Although viral enteropathogens are often detected by these same stool testing panels, their discussion falls outside the scope of this article, as their identification rarely alters management in the immunocompetent adult. This article provides a practical guide for the initial selection and interpretation of stool tests for bacterial as well as parasitic enteropathogens and explores trends in antimicrobial resistance patterns that may impact test selection.

Keywords

Gastrointestinal infections, stool culture, multiplex molecular panels, enzyme immunoassay, *Clostridioides difficile*

Infectious diarrhea affects nearly 179 million people and results in nearly 500,000 hospitalizations in the United States each year.¹⁻³ Most of these cases were attributed to norovirus or were self-limited, with no hospitalization or testing required. In fact, the 2017 Infectious Diseases Society of America (IDSA) guidelines recommend that microbiologic workup be reserved for patients whose clinical picture raises concern for a bacterial etiology or for those patients who are at risk for complications.² Clinically, microbial stool testing is most useful when there is fever, bloody or mucoid stools, severe cramping, signs of systemic toxicity, or the presence of epidemiologic red flags such as recent international travel, known foodborne exposure, daycare contact, hospitalization, recent antibiotic use, or an immunocompromised state.²⁻⁴

Table 1. Overview of Stool-Based Diagnostic Modalities for Bacterial and Protozoal Enteric Infections

Method	Turnaround time, h	Sensitivity	Specificity	Susceptibility data	Key limitations	Detects viable organism?
Stool culture	48-96 h	Low to moderate	High	Yes	Slow; labor-intensive; some organisms are fastidious	Yes
Gram stain	<1 h	Low to moderate	Moderate	No	Useful mainly for <i>Campylobacter</i> ; not routinely performed	Yes (morphology)
Multiplex PCR (eg, GI pathogen panel)	1-2 h	High (>95%)	High (>97%)	No	Cost; detects DNA (not necessarily viable organism); no susceptibilities	No
Toxin EIA (<i>Clostridioides difficile</i>)	1-2 h	Low (~48%-65%)	High (>99%)	No	Low sensitivity; best used in 2-step algorithm with NAAT	Indirect (toxin protein)
GDH EIA (<i>C difficile</i>)	<1 h	High (~93%)	Moderate (~93%)	No	Screening test; detects toxigenic and nontoxigenic strains	No (antigen)
Modified acid-fast stain	1-2 h	Moderate	High	No	For <i>Cryptosporidium</i> , <i>Cyclospora</i> ; requires trained microscopist	Yes (intact oocysts)
Antigen EIA/ DFA (<i>Giardia</i> , <i>Cryptosporidium</i>)	1-2 h	High	High	No	Sensitive only for its 2 targets; multiple specimens advised	No (antigen)
Modified trichrome stain	1-2 h	Moderate	High	No	For microsporidia; must be specifically requested	Yes (spores)
Ova and parasite microscopy	24-72 h	Low to moderate	High	No	Helminth ova and off-panel protozoa; serial specimens needed	Yes

DFA, direct fluorescent antibody; EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; GI, gastrointestinal; NAAT, nucleic acid amplification test; PCR, polymerase chain reaction.

Ordering microbial stool studies in a patient presenting with symptoms suspicious for infectious diarrheal illnesses of bacterial origin, at first glance, may seem to be a straightforward clinical decision. However, the decision whether microbial stool testing is required and which test to order can be challenging owing to the proliferation of available testing options, various clinical scenarios, and downstream implications for managing possible false-positive results.

Broadly, stool diagnostics fall into a few categories (Table 1). Conventional bacterial stool culture has remained the bedrock testing modality for decades. Stool culture provides species-level identification and, importantly, the opportunity for antimicrobial susceptibility testing. Unfortunately, stool culture may take several days to grow and generate an interpretable result, is labor-intensive, and has limited sensitivity for detecting fastidious organisms such as *Campylobacter*.⁵ Alternatively, stool Gram stains can be completed quickly; however, they are generally unhelpful and should only be performed upon special request in the correct patient. Classically, stool

Gram stain with a carbol-fuchsin preparation is useful to detect the “seagull-wing” morphology of *Campylobacter* (reported sensitivity of 66%-94%).⁵ Enzyme immunoassays (EIAs) have variable utility, depending on the specific toxin or antigen that is tested. EIAs are generally fast and specific; however, there are a wide range of sensitivities that depend on the target. For example, *Clostridioides difficile* toxin EIA has a sensitivity of only 50% to 90% depending on the assay.^{6,7}

Finally, the development of multiplex molecular panels in recent years represents a powerful advance in stool testing. For example, the BioFire FilmArray (bioMérieux) gastrointestinal (GI) panel tests for 22 pathogens (bacteria, viruses, and parasites) simultaneously using a single stool specimen, with results that can be obtained in about an hour. The sensitivity and specificity for these panels are usually over 95% and 97%, respectively.⁸ However, multiplex molecular panels come with some challenges in interpretation. Polymerase chain reaction (PCR) tests such as these detect DNA fragments and not necessarily living organisms. Thus, a positive result may reflect

Table 2. Pathogen-Specific Stool Testing

Pathogen	Preferred test(s)	When to test	Key considerations
<i>Clostridioides difficile</i>	NAAT ± toxin EIA (2-step algorithm)	Nosocomial diarrhea; recent antibiotic use	Do not test formed stools; do not repeat within 7 days
<i>Salmonella</i> species	Multiplex PCR; reflex culture for susceptibilities	Bloody/febrile diarrhea; travel; extremes of age	Reflex culture essential for typhoidal species
<i>Campylobacter</i> species	Multiplex PCR; culture for susceptibilities	Acute febrile diarrhea; poultry exposure	Rising fluoroquinolone resistance
<i>Shigella</i> /EIEC species	Multiplex PCR + reflex culture	Bloody diarrhea; day care; MSM; travel	Increasing XDR strains; public health reportable
STEC/ <i>Escherichia coli</i> O157:H7	Multiplex PCR for Shiga toxin type 1/type 2 genes (<i>stx1/stx2</i>); reflex culture on SMAC	Bloody diarrhea without fever	Avoid antibiotics (HUS risk); culture needed for serotyping
<i>Cryptosporidium</i>	PCR; modified acid-fast stain; DFA	Immunocompromised (HIV); prolonged watery diarrhea	Oocysts shed intermittently; multiple samples may be needed
MAC	AFB stool culture; blood cultures	Advanced HIV (CD4 cell count <50 cells/μL)	Disseminated disease common; stool culture slow (weeks)
<i>Yersinia enterocolitica</i>	PCR; CIN agar culture	Right lower quadrant pain mimicking appendicitis; iron overload states	Requires cold enrichment or selective media; often missed on routine culture
<i>Giardia duodenalis</i>	Multiplex PCR; stool antigen (EIA/DFA)	Persistent diarrhea >7 days; surface or recreational water; childcare	Microscopy insensitive; postinfectious IBS common after clearance
<i>Cyclospora cayentanensis</i>	Multiplex PCR; modified acid-fast or UV autofluorescence	Watery diarrhea; spring to summer; with or without travel	Domestically acquired, seasonal; does not reliably self-resolve
<i>Entamoeba histolytica</i>	Multiplex PCR or stool antigen	Dysentery with endemic-area exposure; MSM	Microscopy cannot separate <i>Entamoeba dispar</i> / <i>Entamoeba moshkovskii</i> ; serology if liver abscess
<i>Cystoisospora belli</i>	Modified acid-fast stain; reference PCR	Chronic diarrhea in advanced HIV	Not on multiplex panels; must be specifically requested
Microsporidia	Modified trichrome stain; reference PCR	Chronic malabsorptive diarrhea in profound immunosuppression	Not on multiplex panels; must be specifically requested
<i>Blastocystis</i> and <i>Dientamoeba fragilis</i>	PCR where offered	Rarely indicated as a primary test	Common in asymptomatic hosts; exclude alternatives before treating
Diarrheagenic <i>E coli</i> (EPEC, EAEC, ETEC)	Multiplex PCR	Travel; persistent diarrhea	EPEC rarely meaningful in adults; not distinguishable by routine culture

AFB, acid-fast bacillus; CIN, cefsulodin-irgasan-novobiocin; DFA, direct fluorescent antibody; EAEC, enteroaggregative *E coli*; EIA, enzyme immunoassay; EIEC *E coli*, enteroinvasive EPEC, enteropathogenic *E coli*; ETEC, enterotoxigenic *E coli*; HUS, hemolytic uremic syndrome; IBS, irritable bowel syndrome; MAC, *Mycobacterium avium* complex; MSM, men who have sex with men; NAAT, nucleic acid amplification test; PCR, polymerase chain reaction; SMAC, sorbitol-MacConkey; STEC, Shiga toxin-producing *E coli*; XDR, extensively drug-resistant.

shedding after a recent resolved infection or transient colonization, but not a true infection.⁹ These tests sometimes identify 2 or more organisms, deemed codetections. Codetection rates are particularly prevalent in multiplex molecular panels. One study found codetection in about 24% of positive panels, and their clinical interpretation is often uncertain.¹⁰ Critically, PCR or other multiplex molecular panels provide no data on bacterial susceptibil-

ity, leading to gaps between detection and the ability to provide targeted therapy. To combat this, many laboratories have adopted a reflex-to-culture workflow, whereby an initially positive PCR flags a reportable pathogen such as *Salmonella* or *Shigella*, and then automatically triggers an appropriate stool culture, so that susceptibilities and public health isolates can be obtained.⁹

The current landscape of stool-based diagnostic

options available for common bacterial enteropathogens (*C difficile*, *Salmonella*, *Campylobacter*, *Shigella*, Shiga toxin-producing *Escherichia coli* [STEC], and the diarrheagenic *E coli*), for protozoal and parasitic organisms, and for the less common organisms that may present in immunocompromised patients is explored in the following sections and presented in Table 2.

This article provides a practical guide for the initial selection of a mode of stool testing, discusses challenges in interpretation, offers a practical framework for deciding how a positive multiplex molecular result should influence clinical management, and explores trends in antimicrobial resistance patterns that may impact the selection of specific tests. Notably, viral enteropathogens, such as norovirus, rotavirus, and astrovirus, account for a substantial share of acute gastroenteritis and are included on most multiplex molecular panels. However, these viral enteropathogens are not discussed further in this article, as their detection rarely impacts management decisions in the immunocompetent host.

Common Bacterial Enteropathogens and Their Diagnosis

Clostridioides difficile

C difficile is the most frequently identified cause of health care-associated diarrhea and remains the dominant driver of antibiotic-associated colitis.^{11,12} Yet, despite its prevalence, diagnostic stool testing for *C difficile* probably generates more confusion than for any other GI pathogen.

It is very important to consider some caveats when testing for *C difficile*. First, only unformed stool should be tested for *C difficile*. A positive *C difficile* result in the setting of formed stool almost always identifies colonization, rather than true infection. Thus, many laboratories will not even accept formed stools to test for this reason.^{11,13} Second, testing for *C difficile* should not be repeated within 7 days of a previously negative result. Repeat testing in this circumstance is often of low clinical yield, and the risk of false-positives increases with each round of repeat testing.¹¹

C difficile testing has become progressively sophisticated and nuanced over time, and debate about the ideal method of detection and how to best use available tests is not entirely settled. Stand-alone *C difficile* toxin EIA fell out of favor years ago because its sensitivity is low (ie, only 48%-65%) and thus misses roughly half of true infections.^{6,7} Many laboratories now use some form of a multistep algorithm to detect *C difficile*. The most supported algorithms recommended by the IDSA, European Society of Clinical Microbiology and Infectious Diseases, and American College of Gastroenterology are: (1) a reverse 2-step approach where a nucleic acid amplification test (NAAT) such as PCR is performed first and,

if positive, then the reflexed toxin EIA is performed for confirmation; and (2) a forward algorithm starting with a glutamate dehydrogenase antigen screen, followed by toxin EIA, with NAAT used to arbitrate discordant results.^{11,14-17} Both strategies try to combine the sensitivity of molecular testing with the specificity of toxin detection associated with true infections.

One of the most challenging, and unfortunately quite common, scenarios is for the patient with NAAT-positive but toxin EIA-negative (NAAT+/EIA-) results. A 2025 meta-analysis by Tansarli and colleagues found that although rates of mortality and major complications were comparable between NAAT+/EIA- and NAAT+/EIA+ patients, rates of recurrence and of severe *C difficile* infection were significantly lower in NAAT+/EIA- patients.¹⁸ With this in mind, it is very hard for clinicians to ignore a positive molecular test result. Approximately 73.4% of these NAAT+/EIA- patients ended up receiving treatment, although there was no difference in clinical outcomes in this analysis compared with patients left untreated. When to treat for *C difficile* in this scenario depends on the clinical context and after multidisciplinary discussions. If a patient has a clinically worsening course, other supportive factors such as colonic distention or ileus, or imaging findings demonstrating colonic wall thickening, then it is more than reasonable to treat a NAAT+/EIA- patient for *C difficile*. The principle is that a toxin EIA result alone should not dictate treatment.^{18,19}

Salmonella

Nontyphoidal *Salmonella* accounts for an estimated 1.35 million infections per year, making it one of the most common bacterial foodborne pathogens in the United States.¹ When stool testing is indicated (ie, a patient with moderate to severe disease characterized by bloody diarrhea associated with fever, nosocomial settings, persistent diarrheal illnesses, or in immunocompromised patients), multiplex molecular panels are an effective modality, with good sensitivity and specificity, and results can be obtained in a few hours. However, reflex from positive PCR results to stool culture is important because it provides not only guidance on antimicrobial selection—particularly as fluoroquinolone and extended-spectrum cephalosporin resistance in *Salmonella* species is on the rise—but also helpful information with local public health implications.^{20,21} Patients who are immunocompromised, elderly, or neonatal or who have inflammatory bowel disease or hemoglobinopathies are at elevated risk for invasive disease and merit a lower threshold for both testing and treatment.²⁰ Not all laboratories will reflex to culture by default; therefore, clinicians should know the workflow of their laboratory to better guide evaluation and ensure culture is sent to obtain this epidemiologic information.

Campylobacter

Campylobacter species, primarily *Campylobacter jejuni*, are prevalent sources for infectious gastroenteritis, with several studies demonstrating a higher incidence than *Salmonella*, *Shigella*, and enterotoxigenic *E coli* (ETEC). Consumption and/or handling of raw or undercooked poultry is the most common source.^{22,23} *C jejuni* is a fastidious pathogen with complex nutritional requirements that make it challenging to culture. Successful culture requires microaerobic conditions, 42°C incubation for several days, and the use of selective media.⁵ Given these demanding culture requirements, PCR-based testing has greatly improved the diagnosis of *Campylobacter* infection.^{8,24} Interestingly, rapid presumptive diagnosis can also be obtained using a Gram stain of a fresh, unpreserved stool sample with carbol-fuchsin counterstain. This technique can identify pathognomonic curved, seagull-shaped gram-negative rods with a sensitivity reportedly as high as 94%.⁵ However, Gram stain is not routinely performed, and the typical clinical context usually favors sending multiplex molecular panels. Again, with fluoroquinolone resistance rising, reflex to stool culture for further sensitivity testing is important in patients with suspected *Campylobacter* infection. Analysis of cases from the Foodborne Diseases Active Surveillance Network (FoodNet) linked to the National Antimicrobial Resistance Monitoring System found that ciprofloxacin resistance among *Campylobacter* isolates in the United States rose from 24.5% in 2005 to 2016 to 29.7% in 2017 to 2018, with a parallel rise in extensively drug-resistant (XDR) isolates, while erythromycin resistance remained low at 3.3%.²⁵ The analysis also showed that resistance is substantially more common among isolates from patients with recent international travel than among domestically acquired infections. Macrolides therefore remain the preferred empiric agent when treatment is indicated, with fluoroquinolones reserved for isolates that are confirmed to be sensitive.

Shigella

The classic clinical presentation of *Shigella* infection is bloody, mucoid diarrhea with fever and tenesmus. *Shigella* is easily transmissible through fecal-oral routes between close contacts or through contaminated water or food, as the minimal infective dose is reportedly as low as 10 organisms.²⁶ Shigellosis can be best diagnosed using multiplex molecular panels. On these panels, *Shigella* shares a target with enteroinvasive *E coli* (EIEC) as both organisms carry the invasion plasmid antigen H (*ipaH*) gene. Thus, a positive result is often reported as *Shigella*/EIEC. As discussed previously, stool culture is needed to differentiate the two, and it is essential for a positive multiplex molecular result to reflex to culture for proper identification and susceptibility testing.⁸ XDR *Shigella* has become increasingly more

prevalent, particularly *Shigella sonnei* strains that are simultaneously resistant to fluoroquinolones, azithromycin, and third-generation cephalosporins.^{21,26}

Shiga Toxin–Producing E coli and E coli O157:H7

STEC, including the O157:H7 serotype, has a hallmark clinical presentation with hemorrhagic colitis (often without a fever) and can be complicated by hemolytic uremic syndrome (HUS), particularly in young children.²⁷ The diagnosis requires detecting Shiga toxin either via the presence of the Shiga toxin–encoding genes type 1/type 2 (*stx1/stx2*) on multiplex molecular panels or via Shiga toxin EIA. Stool culture using sorbitol-MacConkey (SMAC) agar identifies O157:H7 specifically; however, SMAC will not grow non-O157 strains, which can account for 20% to 50% of all STEC infections.²⁸ Importantly, antibacterial treatment in patients with STEC is contraindicated. A review article published in *New England Journal of Medicine* found that antibiotics may increase the risk of HUS, with the suggested mechanism being that antibiotics promote rapid toxin release from dying bacteria.²⁹ Because of this phenomenon, timely diagnosis with a multiplex PCR panel can reduce complications and improve patient care.

Diarrheagenic Escherichia coli

In addition to STEC, current multiplex molecular panels detect several additional diarrheagenic *E coli* subtypes, including enteroaggregative *E coli* (EAEC), enteropathogenic *E coli* (EPEC), ETEC, and EIEC. Collectively, these targets generate a disproportionate share of positive panels. Each pathogen is defined by virulence genes, rather than by species-level identification, and each carries a different pretest probability depending on host and exposure. Notably, none of the diarrheagenic *E coli* pathotypes are distinguished by routine stool culture, as stool culture will report normal enteric flora regardless of which virulence genes are present.³⁰

ETEC is the classic cause of travelers' diarrhea and is the most clinically actionable of the *E coli* subtypes. It should be suspected in any returning international traveler with watery diarrhea, and ETEC remains an uncommon cause of domestically acquired illness in adults. EAEC is likewise associated with travel and persistent diarrhea; however, it is also detected in asymptomatic individuals. Therefore, a positive result for EAEC should be weighed against symptom duration and exposure history, and the patient should not be treated automatically. EIEC shares the *ipaH* target with *Shigella* and is reported as *Shigella*/EIEC, as discussed earlier.

EPEC is among the most frequently reported and least meaningful panel results in adults. EPEC is principally found in children living in low-resource settings. In a study of patients with cancer and diarrhea evaluated

by fecal multiplex molecular panels, diarrheagenic *E coli* pathotypes (EPEC most notably) were commonly detected without clear signs of causation of symptoms.³¹ In an adult with an isolated EPEC detection, the appropriate response should often be to consider alternative explanations rather than to treat EPEC specifically. A repeatedly positive result in a symptomatic patient with no alternative diagnosis may warrant further investigation; however, a single detection should not by itself drive antibiotic treatment.

Travel History and Test Selection

Travel history is one of the most useful pieces of information available when selecting a stool test. Uncomplicated travelers' diarrhea is generally self-limited, and expert guidelines support empiric therapy, most commonly single-dose azithromycin with loperamide and rehydration, without an etiologic workup.³² Diagnostic testing should be pursued in travelers with dysentery, high fever, or systemic toxicity, in an immunocompromised host, or in those with persistent symptoms.

The duration of symptoms can help better predict potential pathogens. Early symptoms can generally be attributed to bacterial pathogens, particularly ETEC, EAEC, *Campylobacter*, *Shigella*, and *Salmonella*. As the duration of illness extends beyond 1 week, a protozoal infection caused by *Giardia*, *Cyclospora*, *Cryptosporidium*, or *Entamoeba histolytica* becomes progressively more likely.^{4,33} Testing in these circumstances often changes management, as untreated protozoal infections typically have prolonged symptoms.

Geography may also be helpful for selecting potential pathogens. Fluoroquinolone-resistant *Campylobacter* is considerably more common in returning international travelers than in domestically acquired infection, which argues for the choice of macrolides when empiric therapy is given.²⁵ *E histolytica* is generally found in returning travelers from endemic areas of the Indian subcontinent, Central and South America, sub-Saharan Africa, and Southeast Asia.

Protozoal and Parasitic Enteropathogens

Protozoa are the pathogens most often considered too late into the infectious workup and are also often ordered incorrectly. There are important caveats for the optimal use of stool testing for protozoal infections. First, the duration of illness is the most important factor to consider, as diarrhea persisting beyond 7 days (and particularly beyond 14 days) substantially raises the probability of a protozoal cause. Second, the multiplex molecular panel covers only a narrow range of potentially relevant organisms. The BioFire FilmArray GI panel, for example, includes only

4 (*Cryptosporidium*, *Cyclospora cayetanensis*, *E histolytica*, and *Giardia duodenalis*) of the 22 total parasitic targets.⁸ Other parasites discussed in this section require separate and specifically requested testing.

Giardia duodenalis

G duodenalis (also known as *Giardia lamblia* or *Giardia intestinalis*) is the most identified intestinal protozoan in the United States. Transmission is fecal-oral, classically through untreated surface water but also through recreational bodies of water, childcare settings, and person-to-person contact. The classic presentation is watery diarrhea, bloating, malodorous stools, weight loss, and prominent postprandial symptoms. Stool antigen detection by EIA, direct fluorescent antibody (DFA), or PCR substantially outperforms microscopy. Conventional ova and parasite examination of a single specimen has poor sensitivity because cyst shedding is intermittent and the organism burden may be low. Thus, molecular testing of a single specimen is more sensitive than the sequential specimens historically required for microscopic detection.^{33,34} In laboratories in which a *Giardia* antigen assay rather than a molecular panel is preferentially used, multiple specimens collected on separate days remain the best approach to optimize the diagnostic yield.

Giardiasis also remains relevant to the gastroenterologist after the organism has been cleared. Long-term follow-up of the 2004 Bergen waterborne outbreak found that irritable bowel syndrome (IBS) and chronic fatigue were still significantly more prevalent among exposed individuals than among matched controls up to a decade later.³⁵ Persistent symptoms following documented and treated giardiasis should therefore not automatically prompt repeated courses of antiprotozoal therapy. Rather, postinfectious IBS is the more common explanation, and secondary lactose intolerance accounts for a further proportion of symptomatic individuals. Although genuinely treatment-refractory, giardiasis re-infections do occur; these are less common and warrant confirmatory testing before initiating repeated antimicrobial treatment.

Cyclospora cayetanensis

The characterization of *C cayetanensis* as an infection of travelers returning from Latin America is no longer accurate. Cyclosporiasis is now a domestically acquired and strongly seasonal infection in the United States.³⁶ Cases typically cluster in spring and summer, and outbreaks have been identified and investigated nearly every year since the mid-1990s. The implicated mode of infection is often from imported produce from Mexico and central America. In 2018, two multistate outbreaks alone accounted for 761 laboratory-confirmed illnesses, one of them traced to prepackaged vegetable trays sold in the Midwest.³⁶ More

recently, a large cyclosporiasis outbreak occurred in the United States with nearly 20,000 laboratory-confirmed cases reported after May 1, 2026 by the Centers for Disease Control and Prevention.³⁷ A patient in the United States with watery diarrhea between late spring and late summer is an appropriate candidate for *Cyclospora* testing whether or not they have recently traveled. Clinically, cyclosporiasis is notable for prolonged and often relapsing watery diarrhea with anorexia, weight loss, and marked fatigue, and it does not reliably resolve without treatment. *Cyclospora* is included on the BioFire FilmArray GI panel.⁸ Where molecular testing is unavailable, diagnosis rests on modified acid-fast staining or UV autofluorescence microscopy, and the oocysts are readily missed by a laboratory that has not been directed to specifically look for them.³³

Entamoeba histolytica

E histolytica must be distinguished from *Entamoeba dispar* and *Entamoeba moshkovskii*, which are morphologically identical but nonpathogenic—a distinction microscopy cannot make. Either stool antigen detection by EIA, DFA, or PCR can reliably make the distinction, and molecular testing of a single specimen is an acceptable basis for diagnosis.^{33,34} The older practice of requiring 3 specimens on separate days before accepting a negative result reflects the limitations of microscopy and should not be carried over to molecular testing. Serial specimens do remain relevant to conventional ova and parasite examination for helminth ova, where intermittent shedding is the limiting factor.

Amebiasis should be considered in patients with dysentery who have lived in or traveled to endemic areas of the Indian subcontinent, Central and South America, sub-Saharan Africa, and Southeast Asia, and in men who have sex with men. When amebic liver abscess is suspected, serology is a more useful test, as stool studies are frequently negative in extraintestinal disease.

Blastocystis and Dientamoeba fragilis

Blastocystis species and *Dientamoeba fragilis* occupy a different category: organisms that are frequently detected and rarely actionable. Both are common in asymptomatic individuals, and molecular assays that include them detect them at high rates. Whether either is a genuine cause of GI symptoms remains contested, and the evidence base is limited by the difficulty of assembling appropriate control groups.³⁸ A population-based case-control study found that the prevalence of both intestinal parasites was no greater among individuals with IBS than among controls.³⁹ In an immunocompetent adult, detection of either organism in a patient with diarrhea should prompt evaluation for an alternative explanation such as celiac disease, inflammatory bowel disease, bile acid diarrhea, carbohydrate malabsorption, or a disorder of gut-brain

interaction, rather than empiric antiprotozoal therapy. However, a therapeutic trial may be reasonable in a patient with persistent compatible symptoms and after these alternative diagnoses have been considered.

Uncommon Infections and Special Populations

The standard GI multiplex molecular panels cover many of the most common pathogens. Immunocompromised patients such as patients with HIV/AIDS, transplant recipients, or patients receiving immunotherapy/chemotherapy, however, are at increased risk for infection from atypical pathogens. Immunosuppressed patients also often present with more severe disease, and they may have atypical presentations of more common infections.^{2,40} For these patients, IDSA guidelines recommend sending the typical bacterial pathogen multiplex molecular panel as well as an expanded testing panel that includes parasites (*Cryptosporidium*, *Cyclospora*, *Cystoisospora*), fungi (microsporidia), and mycobacteria.²

Cryptosporidium

Cryptosporidium is a protozoan that causes profuse watery diarrhea in an estimated 748,000 people annually, although most cases go unreported, as the infection is usually self-limited in immunocompetent hosts.⁴¹ However, this infection can be devastating in patients with advanced HIV (particularly in those with CD4 cell counts below 200 cells/ μ L),^{42,43} as it can become chronic and life-threatening. PCR is the most sensitive test, although diagnosis can also be made with modified acid-fast staining, DFA, or antigen EIA.⁴² The BioFire FilmArray panel currently includes screens for *Cryptosporidium*. Importantly, because oocyst shedding can be intermittent, a single negative stool does not completely rule out *Cryptosporidium* infection. If clinical suspicion remains high, multiple specimens should be sent on separate days.^{8,41}

Mycobacterium avium Complex

Disseminated *Mycobacterium avium* complex (MAC) was a significant burden in patients with HIV in the pre-antiretroviral therapy (ART) era, and it remains relevant in patients with CD4 cell counts below 50 cells/ μ L. It usually presents more insidiously with chronic diarrhea, weight loss, fevers, and abdominal pain. Interestingly, the diagnosis is often made from blood acid-fast bacillus (AFB) cultures rather than from stool.⁴⁴ Stool AFB culture may detect MAC but typically takes 2 to 6 weeks to result as positive. With widespread use of ART and specific MAC prophylaxis, disseminated disease is far less common than it once was. Nonetheless, this illness has not disappeared, and it must be considered in the appropriate setting, in

particular in patients who present late into an HIV illness or who had been previously lost to follow-up.^{40,44}

Yersinia enterocolitica

Yersinia enterocolitica is not typically considered in most differential diagnoses for infectious diarrhea. The classic presentation for *Yersinia* is right lower quadrant pain with mesenteric adenitis, often mimicking appendicitis. It is most associated with iron overload states and the consumption of pork products (particularly chitterlings, or chitlins, made from a pig's small intestines).⁴⁵ Standard stool cultures will miss *Yersinia* unless the laboratory uses cold enrichment or specific culture plates with cefsulodin-irgasan-novobiocin agar. Thus, multiplex PCR panels that include *Yersinia*, like the BioFire FilmArray panel, have significantly improved detection.⁸

Cystoisospora belli* and *Microsporidia

Cystoisospora belli and microsporidia, principally *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, cause chronic, voluminous, malabsorptive diarrhea in patients with advanced HIV or other profound deficits in cell-mediated immunity. Neither organism appears on standard multiplex molecular GI panels. *Cystoisospora* is detected by modified acid-fast staining and the microsporidia by modified trichrome (chromotrope) staining or calcofluor white. Reference laboratory PCR is available for both at some centers.³² Because these studies must be ordered explicitly, the practical approach requires notification of the testing laboratory that workup is occurring for chronic diarrhea in a severely immunocompromised patient.

Less Common Pathogens Still Worth Considering

Two further organisms deserve mention for specific clinical scenarios. *Vibrio* species are classically seen after raw seafood consumption or other open saltwater exposure and require thiosulfate-citrate-bile salts-sucrose agar for recovery on stool culture. Thus, the laboratory should be notified in advance whenever *Vibrio* is a consideration. *Plesiomonas shigelloides* and *Aeromonas* species appear on some panels and share a similar association with water and seafood exposure, although their detection in an immunocompetent adult is of unclear clinical importance. *C. cayetanensis*, *C. belli*, and *E. histolytica* were discussed earlier.^{2,5,8}

Interpreting the Positive Multiplex Molecular Panel Result

The practical difficulty with multiplex panels lies less in the sensitivity of the assay than in the clinical actionability of some positive results. First, current multiplex PCR

assays are qualitative rather than quantitative. They report whether nucleic acid is present above a minimal detection threshold, but do not reflect the organism burden, which is often an important variable that separates true infection from passive carriage. Although quantitative PCR panels exist in research settings and could be quite helpful in clinical settings to help distinguish infection from carriage states, this modality of testing has not yet reached routine practice. Second, DNA detection does not imply viability of the detected organism. Nucleic acid persists after an organism has been cleared, and thus a positive result may reflect a resolved infection with ongoing shedding rather than active disease.⁹ Third, transient passage and asymptomatic colonization are common issues, and thus the detection of an organism by PCR may correctly indicate the organisms' presence in the gut but without that same organism functioning as the cause of the patient's illness. Reflex culture data illustrate this point indirectly: in one implementation study, culture reproduced panel-positive results for the major bacterial pathogens in only 56% to 78% of cases, and 2 or more organisms were codetected in 24% of positive panels, most often involving EPEC, EAEC, ETEC, and STEC.¹⁰

Practically, a general clinical framework for interpreting positive multiplex molecular panel results by asking 2 basic questions: (1) Does the clinical presentation (symptom profile, factoring exposure history and duration of illness) match the clinical manifestations known to be associated with the detected organism? and (2) Would treatment of the detected organism change the trajectory of the diarrhea illness? If the answer to these questions is no, then the positive multiplex molecular panel result should not prompt treatment, and the clinical course simply should be monitored. If the answer to either or both of these questions is yes, then appropriate treatment should be offered.

Emerging Challenges

The epidemiologic patterns of infectious diarrhea are shifting. FoodNet surveillance data have shown a rising incidence of non-O157 STEC (with fewer cases of O157 STEC/HUS) and *Cyclospora* infections in the United States. This rise in incidence may be attributed to better diagnostic tools (more efficient at finding cases that were missed using older methods) and to globalized food supply chains, which act as vectors, introducing pathogens to new populations.^{22,46} This highlights the need for the continued monitoring of local and global trends in foodborne illnesses to help inform practice and monitor for outbreaks.

As mentioned, antimicrobial resistance (AMR) in GI pathogens is an emerging challenge. An analysis published in *Lancet* in 2022 attributed 1.27 million global deaths

directly linked to bacterial AMR in 2019.⁴⁷ Among the enteric organisms, the emergence of fluoroquinolone-resistant *Campylobacter*, XDR *Shigella*, and multidrug-resistant nontyphoidal *Salmonella* are the most concerning trends. In practice, this suggests that clinicians should be wary of empiric treatment paradigms. Instead, targeted treatments should be tailored to susceptibility patterns revealed from stool culture.^{21,26,27}

Finally, the rapid results and sensitive nature of stool PCR testing have introduced new challenges in clinical care. These multiplex molecular tests have sensitivities usually above 95%, but they can commonly identify positive results that do not require treatment. The PCR tests identify DNA, not necessarily living organisms, meaning that they can often result positive from postinfectious shedding, colonization, or even dual infections with unclear pathogenicity.^{9,13} Polage and colleagues made this point in their 2015 paper in *JAMA Internal Medicine*, and claimed that a substantial proportion of PCR-positive *C difficile* results did not represent clinically meaningful infection.¹³ The evidence on the downstream effects of panel implementation is mixed. Implementation studies have reported shorter time to appropriate therapy, reduced ancillary testing, and shorter length of stay.¹⁰ The same data, however, show that a substantial proportion of panel-positive results are not reproducible by culture and that codetections are common, both of which create opportunities for treatment that the result cannot justify. The benefit of a rapid and sensitive panel is therefore contingent on the physician's ability to interpret the result and implement thoughtful management. Institutions need stewardship programs that couple molecular diagnostics with clear treatment algorithms, reflex-to-culture protocols for reportable organisms, and ongoing clinician education regarding what organisms to treat and in what clinical scenarios.

There are several emerging technologies in this field that may further advance the ability to detect pathogens and their susceptibility to antibiotics. Metagenomic next-generation sequencing panels can identify up to 176 targets (bacteria, viruses, parasites, virulence factors, and AMR genes), although cost and bioinformatic complexity still limit their broad-based use.⁴⁸ Point-of-care molecular devices continue to shrink in both size and price. Additionally, some groups are working to integrate resistance gene detection, such as extended spectrum β -lactamase (*ESBL*) genes, *qnr* (plasmid-mediated quinolone resistance) determinants, or *mcr* (colistin resistance) markers, directly into multiplex PCR panels.^{49,50} As these technologies advance, they will potentially close the gap between rapid molecular identification and the susceptibility information that currently requires culture and time. Although more work is needed in this area, the future for implementing such rapid and precise stool testing is quickly approaching.

Conclusion

Currently available diagnostic tools for assessing infectious diarrhea are better than they have ever been, and it is fair to say that multiplex PCR panels have been genuinely transformative. These tests are fast, sensitive, and comprehensive in a way that stool culture has never been. However, these new diagnostic tools need to be thoughtfully deployed, and they do not replace stool culture, which remains essential for susceptibility testing, serotyping, and public health surveillance. The two-step *C difficile* algorithm is a useful model for how molecular and immunoassay-based testing can be combined to balance sensitivity and specificity. As is the case with all pathogens discussed, multiplex PCR is only the first step, not the last.

Importantly, not every patient with diarrhea needs to be tested. The immunocompetent adult with mild, watery, afebrile diarrhea and no alarming exposure history will almost certainly get better on their own.²⁻⁴ However, when testing is warranted (patient with bloody stools, high fever, immunocompromised status, recent hospitalization, or recent antibiotic exposure), testing should be done thoughtfully, with an eye toward what result will actually change management. Reflex to culture for susceptibility assessment should accompany positive molecular results for *Salmonella*, *Shigella*, and *Campylobacter*. *C difficile* results should be interpreted through a clinical lens and not treated reflexively. As resistance patterns continue to evolve, stool culture remains an essential reflex test to guide treatment. When diarrhea has persisted beyond 1 or 2 weeks, it is important to consider whether there are risk factors for parasitic infection and recognize that these microbes often require specifically requested tests that are not necessarily included in standard multiplex PCR panels.

Disclosures

Dr VonAxelson has no relevant conflicts of interest to disclose. Dr Levinthal is a consultant for Takeda Pharmaceuticals, Azurity Pharmaceuticals, and Satsuma Pharmaceuticals, and he has served on an advisory board for Gemelli Biotech.

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