Advanced Techniques for Detection and Identification of Microbial Agents of Gastroenteritis

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KEYWORDS

• Gastroenteritis • *Clostridium difficile* • Multiplex polymerase chain reaction
• Suspension array • Next-generation sequencing

KEY POINTS

- Current laboratory methods for diagnosis of gastroenteritis are laborious, have long turnaround times, and can be insensitive.
- Advancements in genomic and proteomic technologies have provided a variety of tools that are being adopted for clinical diagnostics.
- Several nucleic acid amplification–based assays have already been adopted for in vitro diagnostic use while next-generation sequencing is being explored for clinical microbiology applications, with cutting-edge proteomic technologies not far behind.
- These technologies are predicted to provide rapid and accurate diagnosis of infectious gastroenteritis, resulting in improved patient care.

INTRODUCTION

The role of diagnostic microbiology in gastroenterology is to determine whether suspected pathogenic microorganisms are present in test specimens collected from stool, blood, tissue, and other secretions of patients, and, if present, to identify them.[1,2] The gastrointestinal system, in health or disease, is a microbial milieu of unsurpassed variety and complexity. It varies in degree of colonization from the "buggiest" parties

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of the body, at both ends, to the nearly sterile environment of the small intestine and accessory glands. A community of at least 400 distinct species of bacteria, fungi, and protozoa has been identified in the resident flora of the normal gastrointestinal tract, and many more continue to be discovered by molecular approaches, especially next-generation sequencing (NGS) techniques.[3,4] The detection and differentiation of pathogenic among this milieu of commensal flora represents a major challenge to the clinical laboratory.[5]

Infectious diarrhea continues to be a worldwide problem and accounts for more than 2 million deaths annually.[6–8] For example, in the United States it is estimated that there are between 211 and 375 million episodes of acute diarrhea each year, and such episodes are responsible for more than 900,000 hospitalizations and 6000 deaths annually.[9,10] In addition to *Salmonella*, *Shigella*, and enterohemorrhagic *Escherichia coli* (EHEC), the list of potential enteric bacterial pathogens has been expanded to include microorganisms such as *Campylobacter jejuni*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, and *Vibrio* species. *Clostridium difficile* is one of the leading causes of infectious antibiotic-associated diarrhea and pseudomembranous colitis worldwide. This fact is illustrated by the increased incidence and severity of *C difficile* infection, suggesting the emergence of a new hypervirulent strain.[11] Acute viral gastroenteritis is the second most common infectious disease worldwide.[8,12] Known enteric viral pathogens include rotavirus, norovirus, astrovirus, and adenovirus serotypes 40 and 41.[13–15] In recent years the number of reported gastroenteritis outbreaks of suspected viral etiology has increased, hence the need for rapid, sensitive, and reliable diagnostic assays.[14,15] Other parasites (*Giardia*, *Entamoeba histolytica*, and *Cryptosporidium*) have been reported to be pathogens causing gastroenteritis.[16,17]

Timely and accurate identification of suspected pathogens that are present in the stool specimens from patients with signs and symptoms of gastroenteritis is still a challenge in the clinical laboratory. Microscopic examination is conventionally required for parasite identification. An ova and parasite (O&P) examination for identification of the parasite form requires a trained technologist. However, in some cases microscopic examination is limited in distinguishing species. For example, one could not distinguish *E histolytica* (pathogenic species) from other nonpathogenic species, such as *Entamoeba dispar*.

Examination by electron microscopy (EM) can be used for a range of viruses, including adenovirus, norovirus, and rotavirus, but the method is cumbersome and requires significant expertise. EM was initially the only method for norovirus diagnosis, but its use has been limited owing to its low sensitivity and specificity. Various stool antigen tests have been available for the detection of different gastroenteritis pathogens. Although most antigen tests are simple and rapid, they usually lack sensitivity and specificity. Several comparison studies have shown that nonmolecular tests, such as the glutamate dehydrogenase (GDH) antigen test and enzyme immunoassay (EIA) for toxins A and B, are either less sensitive or less specific than molecular testing for *C difficile* infection detection.[18–20]

Bacterial stool cultures remain the mainstay of the laboratory evaluation of diarrheal illnesses. Selective agars are used to facilitate the isolation of suspicious enteric bacterial pathogens. Pure cultures obtained subsequently permit characterization and identification by morphologic and biochemical characteristics. These conventional methods, however, are time consuming, labor intensive, and require experienced clinical microbiologists. Identification and confirmation of suspicious colonies is highly nonspecific, and places a heavy burden on the laboratory in terms of biochemical and serologic testing.[21–23] Stool culture–based enteric bacterial