



Review article

Viral pathogen detection using multiplex gastrointestinal molecular panels: The pros and cons of viral target inclusion

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ABSTRACT

Diagnosis of gastrointestinal infections has been revolutionized by the development of in vitro diagnostic (IVD) multiplex molecular panels for the detection of viral nucleic acids. In addition to a high degree of accuracy, these panels are commercially available and relatively simple to perform in the clinical laboratory. However, use of these panels must be carefully considered owing to the laboratory costs of the test, limited reimbursement, and potential for overuse. In this review from the Pan American Society for Clinical Virology, we focus on the viral components of GI multiplex panels (GIPs), presenting a brief overview of pathogens included on most panels and a discussion of advantages and challenges of the inclusion of viral targets on GIPs that should be considered before implementation in the clinical laboratory.

1. Background

Laboratories implementing a gastrointestinal multiplex panel (GIP) have many options, with several more panels in development. Choosing the best panel for a laboratory requires consideration of many factors, including which targets are included and how many targets are on the panel. A previous publication from the Pan-American Society of Clinical Virology reviews the available panels and considerations for implementation [1]. In this review, we focus on the viral causes of gastroenteritis and discuss the pros and cons of the inclusion of these viral targets on GIPs.

2. Review of viruses included on GIPs

Norovirus is a single-stranded positive sense non-enveloped RNA virus belonging to the family *Caliciviridae*. It is estimated that 658 million cases occur yearly, with 200 million cases in children <5 years of age [2]. Norovirus infections are typically characterized by sudden onset of nausea, vomiting, and diarrhea lasting 1–3 days. Severe disease may occur in individuals with underlying medical comorbidities, immunosuppression, or extremes of age. Asymptomatic infections may occur, and individuals may shed substantial levels of virus, posing an infection risk [3]. Patients receiving hematopoietic stem cell transplants or solid

organ transplants are at particular risk of severe disease and prolonged RT-PCR positivity due to persistent viral shedding for months after initial infection [4]. Due to the high viral loads (10^5 – 10^8 cDNA copies/g) in stool from infected individuals, norovirus is frequently associated with transmission in close contact settings such as schools, health care environments, military barracks, and cruise ships. Strict infection control measures and enhanced environmental cleaning are key to controlling outbreaks [4]. As norovirus cannot be propagated in cell culture, alternative modalities for detection such as enzyme immunoassay, rapid antigen assays, and molecular amplification/detection are the mainstays for diagnosis.

Rotavirus is a double-stranded RNA virus in the family *Reoviridae*, which can cause severe gastroenteritis in children under 5 years old [5]. Of the GI viruses discussed herein, rotavirus is the only one with oral vaccines available (RotaTaq®, licensed in 2006, and Rotarix®, licensed in 2008) [5]. Despite accessibility of an effective oral vaccine, data from 2013 showed there are still more than 200,000 deaths annually due to rotavirus in low-income countries [6]. Rotavirus causes a non-bloody diarrhea, accompanied by malaise, fever, and vomiting. The seasonality of rotavirus cases is not well-defined but in temperate regions, peaks of infection are seen in winter months [7]. The most common methods for laboratory diagnosis are immunoassays that detect viral antigen in stool, stand-alone RT-PCR tests, or multiplex molecular

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assays.

Human Adenoviruses (HAdVs) are non-enveloped viruses with a double stranded DNA genome. Unique among gastrointestinal viruses, HAdV has more than 100 types, not all of which are associated with gastrointestinal disease. HAdV 40 and 41 are the second most common cause of gastroenteritis in adults and children under the age of 2 and are the causative agent for 2–15 % of pediatric diarrhea [8]. Infection is nearly ubiquitous, with a seroprevalence of 94 % in the United States [8]. HAdV 41/adeno-associated virus type 2 co-infection has also been associated with pediatric hepatitis [9,10]. Less common gastrointestinal types are associated with appendicitis and intussusception and may be accompanied by upper respiratory symptoms. Of the GI viruses discussed here, HAdV are associated with the fewest number of outbreaks, though patients infected with HAdV are the most likely to report fever [11]. While adenovirus can be detected using viral culture or antigen testing for HAdV 40/41, these methods are not available in most clinical laboratories [12]; today, GI HAdV infections are most often diagnosed via nucleic acid amplification testing.

Human Astrovirus (HAstV) is a non-enveloped virus with a positive-sense, single stranded RNA genome. HAstV is the causative agent for 2–9 % of pediatric diarrhea cases [13]. Infection during early childhood is very common, with incidence peaking from 12 - 17 months of age [14]. Among infected children, approximately 60 % of patients experience both diarrhea and vomiting at infection onset [15]. There are few reports of symptomatic infection in adults, possibly due to the presence of protective antibodies following childhood infection. Like other GI viruses, HAstV has been implicated in daycare center disease outbreaks [16]. While disease is typically self-limiting, viremia and central nervous system infections have been documented in immunocompromised patients. HAstV infection is diagnosed via nucleic acid amplification testing [17].

Another member of the *Caliciviridae* is sapovirus, which is a non-enveloped, positive sense single-stranded RNA virus [18]. Disease due to sapovirus is generally less severe compared to norovirus infections. Acute onset of diarrhea, vomiting, nausea, chills, myalgia, and malaise are commonly reported. Symptoms are generally self-limiting (1–2 days), although fecal shedding may continue for 1–4 weeks. There is currently little evidence of enhanced pathogenesis of sapovirus in susceptible groups such as immunocompromised patients or neonates. Treatment of sapovirus disease is supportive, and hospitalization is rarely needed [18]. Evidence suggests sapovirus is an emerging cause of GI disease in children and is most often seen in children under 5 years old [19]. Fecal-oral transmission has been associated with large food-borne outbreaks. Co-infections of sapovirus and other GI viruses including norovirus, astrovirus, rotavirus, and HAdV have been reported [18]. As commercial assays for sapovirus antigen detection are unavailable, RT-PCR methods are the primary diagnostic methodology.

3. Pros of inclusion of viruses on GI panels

3.1. Epidemiology and public health

Inclusion of viral targets in multiplex GIPs is valuable from a public health standpoint, allowing for identification and tracking of specific viral pathogens and the ability to differentiate between bacterial and/or parasitic etiologies. Use of broad multiplex panels has already elicited significant data on the rate of cases associated with co-infections, the seasonality of infections, and relative rates of disease attributable to viral pathogens [20,21]. GIPs have also been used to quickly identify additional cases during outbreak investigations [22]. The benefits of on-site testing are likely to be even higher for viral pathogens since referral testing would otherwise be required to determine if a patient is infected with the outbreak pathogen. The ease of use and small equipment footprint of many multiplex panels also makes them ideal for use in resource limited settings that may not have access to viral culture, electron microscopy, or more complex manual PCR methods through a

readily available public health infrastructure [23]. Though cost is a strong consideration before use can be widespread, this methodology has potential utility for early outbreak identification and control.

While GIP data will be useful for faster and more accurate outbreak identification, it will also be essential for improving public health resources. By better understanding the incidence of viral agents of gastroenteritis, antiviral and vaccine development resources can be prioritized. The clinical relevance of less recognized viruses (sapovirus, astrovirus) can also be better understood. When new clinical syndromes are associated with known viruses (ex, pediatric hepatitis and its potential association with HAdV), enhanced access to accurate, rapid results can aid in quickly identifying patients who may be affected [24]. Finally, continued incidence data will be essential in monitoring the efficacy of the rotavirus vaccine, how this affects the incidence of other GI pathogens, and the efficacy of any future vaccine(s) for viral gastroenteritis pathogens.

3.2. Improved time to diagnosis and diagnostic accuracy

Given the broad overlap of clinical signs and symptoms, it is challenging to distinguish between various causes of gastroenteritis without definitive diagnostic testing. Information such as time of year, patient age, vaccination status (i.e. rotavirus), travel history, known outbreaks or exposures may be suggestive of potential etiologies but is non-specific. Reliance on individual tests of potential causes, especially if done sequentially, can delay definitive diagnosis. GIPs offer the ability to test for most of the common causes of viral gastroenteritis in a single step, aiding in accurate detection of common pathogens or can rule these out as the cause of infection. Multiple studies show ancillary effects such as potential reduction of unnecessary laboratory testing (e.g., bacterial stool culture), invasive diagnostic procedures (e.g., colonoscopy), and improved therapeutic decision making [21,25]. Ruling in a viral cause has the potential to alleviate the overuse of antibiotics, unnecessary imaging procedures, and costly Emergency Department visits for primary care.

In certain cases, identification of the viral cause may impact infection control practices and inpatient isolation procedures (i.e., Norovirus), or if an outbreak is occurring at daycare or school [26]. Rand et al. demonstrated improvements in infection control practices reducing nosocomial transmission after GIP implementation [27]. In addition, Goldenberg et al. demonstrated a reduction in overall hospital isolation time after implementation of GIP [28]. Inclusion of viral targets on GIPs could therefore be considered an important part of antimicrobial stewardship and outbreak management. A recent database analysis of hospital records from adult outpatients with a diagnosis of acute gastroenteritis indicated lower 30-day cost of care and risk of hospitalization with GIPs, as compared to stool culture, with at least one GI pathogen identified in 54.6 % of patients [29].

3.3. Immunocompromised population and special populations/cases

In the 2017 Infectious Disease Society of America (IDSA) guidelines for management of infectious diarrhea, a broad approach to diagnosis including assessment for bacterial, viral, and parasitic causes was recommended for immunocompromised hosts (ICHs) [30]. GIPs are well-suited for this application due to their array of viral targets and rapid time to result. Based on IDSA guidelines, the Center for Medicare and Medicaid Services (CMS) supports reimbursement of broad-range GIPs (> 12 analytes) for ICH testing, further supporting prudent use of GIPs in ICHs [31].

Various studies support the utility of multiplex GIPs for diagnosis of viral pathogens in ICH populations. In a study by Sobczyk et al., 23 % of HIV patients presenting with diarrhea tested positive for a GI virus that would have not otherwise been identified by a conventional laboratory workup [32]. A study by Cancho et al. demonstrated that GIP testing contributed to the ability to rule out infectious causes of diarrhea in 74

% of 95 ICH hematologic patients, as well as rapid identification of norovirus, rotavirus and HAdV infections that might have gone undetected [33]. As suggested by Liesman et al., the advantages of GIP testing in ICH populations must be balanced against the inability of molecular assays to distinguish DNA/RNA from viable organisms [34]. Persistence of residual viral nucleic acid can lead to positive GIP results in asymptomatic patients, potentially resulting in overtreatment. Results should be evaluated in the context of underlying immunosuppression [34].

Pediatric patients are a special group for which GIPs have been investigated for clinical utility. Currently, no specific clinical guidelines exist endorsing the routine use of GIPs for these patients, as pediatric gastroenteritis is generally self-limiting. Several studies, however, have demonstrated that use of GIPs in pediatric populations can improve time to diagnosis, prevent unnecessary prescription of antibiotics, and can prevent or slow in-hospital transmission [35,36]. Cotter et al. showed that time to result was decreased from 31 h to 4 h [35]. Yoo et al. observed that antibiotic use decreased from 71.8 % to 35.3 % after GIPs were implemented [36]. In the same study, a small cohort were chosen to assess impact hospital isolation procedures [36]. Of those who had a detectable pathogen on a GIP, 78.6 % were identified as norovirus, which requires rapid control measures [36]. Due to the quick identification, 38.5 % of patients were isolated the same day, and 53.8 % were isolated next day [36].

4. Cons of inclusion of viruses on GI panels

4.1. Cost of testing and reimbursement

GIPs are more expensive to run than other common diagnostic methods for gastroenteritis and are billed at a higher rate. This higher cost coupled with the fact that these panels are not appropriate in many clinical scenarios means that coverage by insurance (both private and government) is conditional. There are three common CPT codes used for billing: CPT code 87505 (gastrointestinal pathogen, 3–5 targets); CPT code 87506 (6–11 targets); and CPT code 87507 (12–25 targets) (CPT® (Current Procedural Terminology) | AMA (ama-assn.org)). Inclusion of both bacterial and viral targets means that it is likely CPT code 87506 or 87507 would be billed, which often requires specific ICD-10 codes to justify the use of the larger panels. One option would be to limit multiplex GI panels to bacterial causes that have specific treatments, especially if the number of targets does not exceed five, as these could be billed with CPT code 87505 (gastrointestinal pathogen, 3–5 targets). Policies and coverage requirements for each CPT code varies by plan and are defined in Local Coverage Determination (LCD) or other plan documents (for example, see articles L38229, A56642, A56638, A56596, L39003, A58726, L39226, A58963, L38227, and L37364 from Centers for Medicare and Medicaid Services (CMS)) [31]. Those responsible for submitting laboratory orders should ensure the proper International Statistical Classification of Diseases (ICD)-10 code is submitted as required in plan documents to increase the likelihood for reimbursement. Insurance denials can result in financial burdens for the healthcare system, laboratories, and in some cases, patients. Patients without insurance, or whose insurance does not cover GIPs, can receive large bills that are hard to justify, especially if the cause of gastroenteritis was self-limiting and the test result did not alter patient management. CPT code 87505 (gastrointestinal pathogen, 3–5 targets) tends to be reimbursed more favorably, resulting in reduced healthcare costs.

4.2. Viral detection on GIPs unlikely to affect management of patient

Unlike bacterial targets that have effective, readily available treatments, detection of the viral cause of gastroenteritis is unlikely to impact the management of most patients. This is particularly true for patients seen in the ambulatory setting, where reimbursement is a significant concern. Treatment of viral gastroenteritis is supportive, consisting of correction of electrolytes and dehydration in the majority of cases [30].

For patients presenting with gastroenteritis who are otherwise healthy, no testing is recommended unless symptoms last longer than 7 days or are severe [30]; even in this scenario, ruling out bacterial infection is likely adequate. This approach is recommended by IDSA guidelines, which support testing symptomatic patients for *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *C. difficile*, and STEC only [30]. Testing for viral targets is only recommended when an outbreak is suspected, or a patient is immunosuppressed. For most laboratories, these types of patients will not make up a significant proportion of individuals being tested.

While several studies have shown that GIP use can reduce antimicrobial usage and endoscopy rates, it is unclear if this is due to the ability to rapidly diagnose patients with a viral infection or merely the ability to rapidly exclude bacterial infections [21,25]. Furthermore, this reduction in follow-up procedures may not always be indicative of better resource utilization. If a patient with asymptomatic carriage of a virus is tested using a broad multiplex panel, their symptoms may be mistakenly attributed to the virus rather than a potential non-infectious cause that requires further diagnostic workup. If multiplex panels are restricted to clinically actionable bacterial targets, this will provide adequate information to guide the management of most patients while avoiding confounding diagnoses and potential reimbursement issues.

4.3. Inappropriate use of multiplex panels

The ability to accurately detect a broad range of intestinal pathogens in a relatively simple manner is an attractive diagnostic tool, but potentially misused for the wrong indications. Although specific, molecular-based tests can detect residual nucleic acid for weeks to months after the initial infection has cleared. GIPs are not intended to be used as “tests of cure” [37]. Using GIPs for this purpose can confound diagnosis by potentially masking the true etiology of disease or prolonging inappropriate treatment with antimicrobial or antiparasitic agents. As most community-acquired cases of diarrhea are self-limited and require no treatment, identification of the specific agent is not warranted in most instances [30]. In addition, use of GIPs is not recommended in patients hospitalized ≥ 72 h [38]. Due to the nuances of GIP utilization, implementation of these tests should only be considered through a collaborative effort with clinical services, including education on the use of the test, development of testing algorithms to guide utilization, and ideally, use of clinical decision support tools for ordering via electronic health record (EHR) systems [34,38].

In cooperation with clinical services, laboratories must consider the increased expense of testing and the current limitations on laboratory reimbursement for GIPs. Multiplex panels incur laboratory reagent costs significantly higher than conventional antigen and culture-based tests [34]. A recent study from Moon et al. in an adult outpatient population demonstrated multiplex GI panel testing was associated with higher initial visit costs (difference of \$97.00 USD), but a lower 30-day follow up cost (difference of \$117.00 USD) compared to stool culture [29]. As of October 2022, the Center for Medicare and Medicaid Services (CMS) limits coverage of GIPs to 5 bacterial targets for most outpatients, excluding viral and parasitic targets [39]. Prudent use of patient healthcare dollars in accordance with clinical standards of care should be a strong factor in the recommendation to not utilize broad GIPs for low-risk patients in an outpatient setting.

5. Potential solutions

Although the technology allows for the inclusion of numerous pathogen targets from a single reaction, the high cost of testing, limited insurance coverage, and medical necessity warrants a more thoughtful approach (Table 1). Cost notwithstanding, the epidemiological and public health benefits, improved speed of diagnosis, superior diagnostic accuracy, and benefit for immunocompromised patients would support routine use of large GIPs in the appropriate clinical and epidemiological scenarios. However, laboratory stewardships concerns along with

Table 1

Summary of pros and cons of viral target inclusion and potential solutions.

Pros:
Faster and near-to-patient detection of outbreaks
More complete data for public health and drug/vaccine development
Faster diagnosis or rule-out, leading to fewer antibiotics used and fewer endoscopic procedures
Faster time to proper isolation (when required)
Testing recommended by Infectious Disease Society of America for immunocompromised hosts
Cons:
Poor insurance reimbursement as compared to bacteria-only panels
Increased financial burden for labs, health systems, or patients
Viral testing not recommended for otherwise healthy patients
Over-diagnosis may mask non-infectious causes of gastroenteritis
Misuse of tests as test of cure or in inappropriate populations
Potential Solutions:
Masking targets by manufacturer to create custom panels
Not reporting results in certain patient populations
Small panels labs can mix and match to customize
Flex-based pricing based on CPT code

limited utility of viral results for management of most patients makes a one-size-fits all GIP recommendation impractical. Considerations such as inpatient versus outpatient, immunocompromised versus immunocompetent, patient age, and travel history factor into deciding the appropriate targets. The flexibility to mask targets and create panels specific to the clinical scenario would be a beneficial and practical solution to this issue. Ideally, masking options would be at the instrument software level to blind the result to the operator. Other options would be masking through the Laboratory Information System (LIS), but that may present an ethical dilemma if the operator is able to see the result. Another solution may be smaller panels in clinically relevant groupings that could be ordered alone, or in combination with other small panels depending on the clinical situation. Laboratory stewardship of high-cost assays is critical, and both of these solutions would allow a tailored approach based on the clinical situation. Additionally, vendors may consider flex-based pricing (where the laboratory is only billed for targets that are tested) with subsequent billing by the laboratory with the appropriate CPT code based on target number to improve the acceptance and appropriate utilization of GIPs.

Disclosures/Disclaimers

This Report is a product of the PASCV Clinical Practice Committee (CPC) developed to provide guidance and recommendations to healthcare professionals considering implementation of multiplex molecular tests. It is not clinical advice and does not represent all possible facets of panel utility.

Declaration of Competing Interest

D. Jane Hata and Eleanor Powell report no financial conflicts of interest. M. Starolis is a full-time employee and stockholder at Quest Diagnostics and has served as an unpaid advisor to Roche and BioRad. D. Jane Hata, Eleanor Powell, and M. Starolis are all members of the Pan American Society for Clinical Virology Clinical Practice and Public Policy Committee.

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