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# Systematic application of multiplex PCR enhances the detection of bacteria, parasites, and viruses in stool samples

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**Summary** *Objectives:* To determine whether systematic testing of faecal samples with a broad range multiplex PCR increases the diagnostic yield in patients with diarrhoea compared with conventional methods and a clinician initiated testing strategy.
*Methods:* 1758 faecal samples from 1516 patients with diarrhoea submitted to two diagnostic laboratories were tested for viral, bacterial, and parasitic pathogens by Fast-Track Diagnostics multiplex real-time PCR kits and conventional diagnostic tests.
*Results:* Multiplex PCR detected pathogens in 530 samples (30%): adenovirus (51, 3%), astrovirus (95, 5%), norovirus (172, 10%), rotavirus (3, 0.2%), *Campylobacter jejuni/coli* (85, 5%), *Salmonella* spp. (22, 1%), *Clostridium difficile* (72, 4%), entero-haemorrhagic *Escherichia coli* (21, 1%), *Cryptosporidium* spp. (3, 0.2%), *Entamoeba histolytica* (1, 0.1%), and *Giardia lamblia* (59, 3%). In contrast, conventional testing detected a pathogen in 324 (18%) samples.
*Conclusions:* Using a systematic approach to the diagnosis of gastroenteritis improved diagnostic yield. This enhanced detection with PCR was achieved by a combination of improved detection of individual pathogens and detection of pathogens not requested or unable to be tested by conventional tests. This approach also allowed earlier identification for most pathogens and created a workflow which is likely to adapt well for many diagnostic laboratories.


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## Introduction

Diarrhoeal disease is a leading cause of mortality and morbidity worldwide,[1] and can also place considerable financial burden on health care systems through hospitalisations and complications such as Guillain–Barré syndrome.[2]

The laboratory detection of gastroenteric pathogens still relies on microscopy, culture, and immunoassays.[3] Turnaround times for these tests are variable, but it can take several days to exclude organisms such as *Campylobacter* spp. and *Salmonella* spp.[4] PCR is an established tool for the diagnosis of norovirus infection,[5] and several studies have examined the use of multiplex PCR for the diagnosis of bacterial,[6,7] viral,[8,3] or parasitic[9,10] causes of diarrhoea. These have demonstrated enhanced detection of most pathogens with more rapid turn-around times, and the ability to test for a greater range of targets. The presence of important pathogens such as *Giardia lamblia* has also been demonstrated in samples requesting examination only for bacteria.[11]

We describe the prospective application of combined viral, bacterial and parasite multiplex PCR panels from Fast-Track Diagnostics (FTD), (Fast-track Diagnostics, Junglinster, Luxembourg) on consecutive faeces specimens from patients with diarrhoea. The main aim of the study was to assess whether the systematic application of multiplex PCR would result in an increased diagnostic yield over existing diagnostic testing based on tests routinely performed at our laboratories, and a clinician initiated testing strategy.

## Materials and methods

### Specimens

Sequential faecal specimens submitted from community or hospital sources to the microbiology laboratories of Canterbury Health Laboratories (CHL) or Medlab South (MLS), Christchurch, New Zealand between April and June 2011 were included in the study. At the time, both CHL and MLS were operating from the same facility and were using similar laboratory protocols. Formed specimens and those with insufficient volume for PCR testing and storage were excluded.

All specimens were routinely cultured for bacterial pathogens and, at the request of referring clinicians, were tested for other enteric pathogens (Table 1). In addition, approximately 200 mg of faeces was transferred into 1 mL of Stool Transport and Recovery buffer (STAR, Roche Diagnostics, Auckland, New Zealand) and tested as soon as possible by multiplex PCR. An additional aliquot of unprocessed faeces and the STAR buffer suspension were stored at −80 °C in case further testing was required.

The study was approved by the Upper South B Ethics Committee.

### Nucleic acid extraction

Specimens in STAR buffer were pre-processed by centrifugation at 13,000 rpm for 2 min to remove faecal matter. 60 μL of supernatant was added to 240 μL of NucliSENS lysis buffer (BioMerieux, Sydney, Australia) with 2 μL of internal control (FTD) and extracted using the NucliSENS easyMag (BioMerieux) generic 2.0A protocol. Nucleic acid was eluted into a final volume of 75 μL. Specimens unable to be pipetted due to the presence of mucous were first diluted 1:2 and centrifuged. If still unable to be pipetted, mucous was withdrawn from the specimen. Specimens found to be inhibited after PCR amplification and analysis were diluted 1:10 in STAR buffer and re-extracted as above.

### PCR amplification

PCR was performed according to the manufacturer's instructions using FTD Bacterial gastroenteritis (2 pools); FTD Viral gastroenteritis (2 pools) FTD Parasite gastroenteritis (1 pool) kits and AgPath-ID™ One-Step enzyme (Applied Biosystems, Life technologies, Melbourne, Australia) with

**Table 1** Pathogen detection by conventional methods according to clinician request, multiplex PCR (M-PCR) detection in samples where both methods were applied.

| Pathogen | Conventional positive | % | Multiplex PCR positive | % | Total number tested |
|---|---|---|---|---|---|
| *Campylobacter* spp | 84 | 4.8 | 85 | 4.8 | 1758 |
| *C. difficile* | 15 | 4 | 24 | 6 | 370 |
| Entero-haemorrhagic *Escherichia coli* (EHEC) | 5 | 0.3 | 5 | 0.3 | 278 |
| *Salmonella* spp. | 31 | 1.8 | 24 | 1.3 | 1758 |
| *Shigella* spp. | 0 | 0 | 0 | 0 | 1758 |
| *Yersinia* spp. | 9 | 0.1 | 0 | 0 | 1758 |
| Adenovirus | 0 | 0 | 1 | 2 | 45 |
| Norovirus GII | 36 | 22 | 37 | 22 | 165 |
| Rotavirus | 25 | 6.7 | 1 | 0.3 | 373 |
| *Cryptosporidium* | 1 | 0.1 | 0 | 0 | 774 |
| *E. histolytica* | 1 | 0.3 | 0 | 0 | 295 |
| *G. lamblia* | 23 | 3.0 | 42 | 5.4 | 774 |