Gastrointestinal disease (GI) is a major cause of morbidity and mortality worldwide. GI can be caused by a wide range of infectious agents including viral, bacterial and protozoa. Human viral gastroenteritis can be caused by Noroviruses, Rotaviruses, Adenoviruses, Astroviruses and Sapoviruses. Of these Norovirus is the most commonly isolated agent as the cause of acute viral gastroenteritis (1). According to the CDC 43% of bacterial GI infections are caused by Salmonella, followed by Campylobacter (33%), Shigella (17%), Escherichia coli (4%) and Yersinia (0.9%). Another cause of bacterial GI are hypervirulent strains of Clostridium difficile particularly PCR ribotype 027 (2). Among parasites Giardia intestinalis, Cryptosporidium sp and Entamoeba histolytica are considered the most common and important causes of diarrhea (3) although other species such as Blastocystis hominis have also been implicated in GI disease. Thus the diagnosis of GI can be challenging and involve specialists in microbiology, virology and parasitology.

A universal sample processing method was devised that lysed and simultaneously converted the nucleic acids of bacteria, viruses and protozoan parasites from the primary patient sample. The sample buffer protects the labile RNA species from the harsh conditions required for complete lysis of tough organisms such as Giardia. The procedure comprises a single tube method during which the faecal sample is incubated in extraction buffer for 15 minutes. Samples were then purified using a column based method or automated platforms such as Roche, Qiagen and Thermo. PCR can then be carried out on most real-time instruments including those from Roche, Qiagen, Cepheid, ABI, Biorad and Stratagene. All reagents required for sample to result are included simplifying the method for the end user.

All assays were linear from 10^3-10^ copies and no cross reactivity was observed between individual primers and a larger number of bacterial and fungal non-target species. Over 500 clinical samples have been assessed and compared to conventional techniques such as culture, EIA and microscopy with excellent concordance. The method developed here is therefore suitable to the rapid and sensitive screening of primary patient material for a wide range of common GI pathogens.

### Objective

In order to simplify the detection of causative agents of GI we have developed rapid real time multiplex PCR (mPCR) panels for all major GI pathogens (see Table 1). All assays share a universal sample processing method and incorporate our previously described 3base™ technology (4). Furthermore, conventional GI diagnosis can in some instances take up to 5 days (5) to provide a definitive result. To reduce this time we aimed to produce assays with sample to result turnaround time in as little as 3 hours.

### Results

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### Discussion

The assays developed here may be used as a complete screening system for the diagnosis of all major GI pathogens from primary clinical samples. The assays are simple and employ universal sample preparation conditions thereby streamlining the process of pathogen detection from faecal material. All assays have incorporated controls for sample processing and inhibition to ensure assay robustness and reliability. Assays can be run on virtually all purification and real time instruments found in major hospital and pathology laboratories. Sample to results time is less than 3 hours, allowing for rapid diagnosis facilitating optimal patient management.

### References