Multiplex PCR detection of all major gastrointestinal pathogens employing a novel universal extraction method

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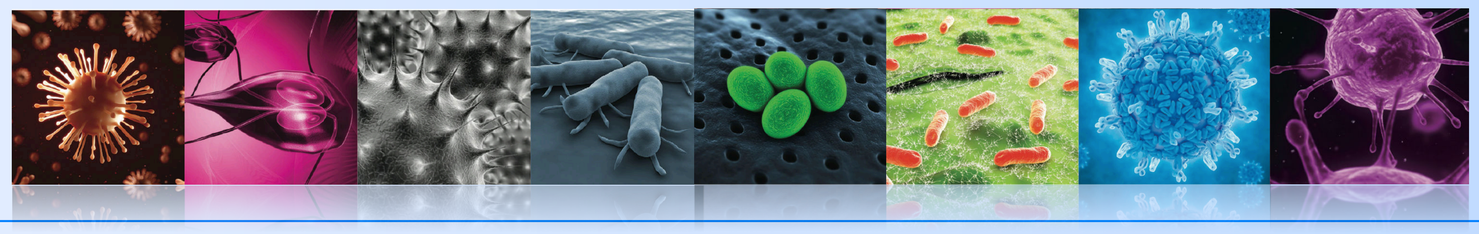
Genetic Signatures



Genetic Signatures

Abstract

Gastrointestinal disease (GI) is a major cause of morbidity and mortality world-wide. GI can be caused by a wide range of infectious agents including viral, bacterial and protozoa. Human viral gastroenteritis can be caused Noroviruses, Rotaviruses, Adenoviruses, Astroviruses and Sapovirus. 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* (4.1%) 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* spp and *Entamoeba histolytica* are considered the most common and important causes of diarrhea (3) although other species such as *Dientamoeba fragilis* and *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.

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.

Materials & Methods

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 from sample to result are included simplifying the method for the end user.

Figure 1. Amplification plots obtained using the *C. difficile EasyScreen*™ detection kit.

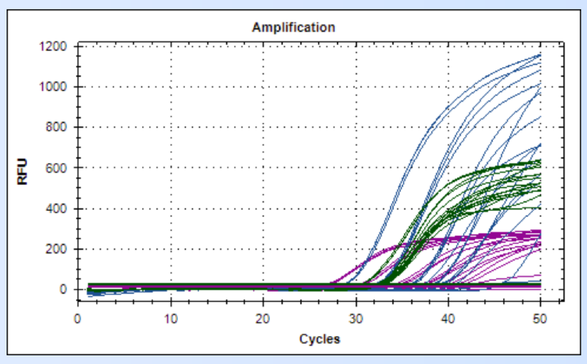


Table 2. Three independent Clinical studies using the *EasyScreen*™ *C. diffiicle* assay.

	Study #1(n=70)
C. difficile Culture+	70 (100%)
EIA*	43 (61.4%)
*EasyScreen C. difficile**	53 (75.7%)

	(n=73)	
	Positive	%
C. difficile Culture+	63/72	88
EIA*	31/72	43
Illumigene*	56/73	76.7
Quik Chek Complete*	35/52	67.3
*EasyScreen C. difficile**	56/73	76.7

	Study #3 (n=74)
Culture Toxin +ve	61/74 (82.4%)
EasyScreen +ve[1]	65/74 (87.8%)
Illumigene +ve[2]	65/74 (87.8%)
Ausdiagnostic +ve[3]	36/69 (52.2%)
Faecal Toxin +ve[4]	33/74 (44.6%)
[1] 61/61 CT positive samples (100%)	
[2] 59/61 CT positive samples (96.7%)	
[3] 36/58 CT positive samples (62.1%)	
[4] 33/74CT positive samples (45.6%)	

*Not toxigenic culture

Results

All assays were linear from 10^6-10 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.

Figure 2. Amplification plots obtained using the *EasyScreen*™ Parasite assay.

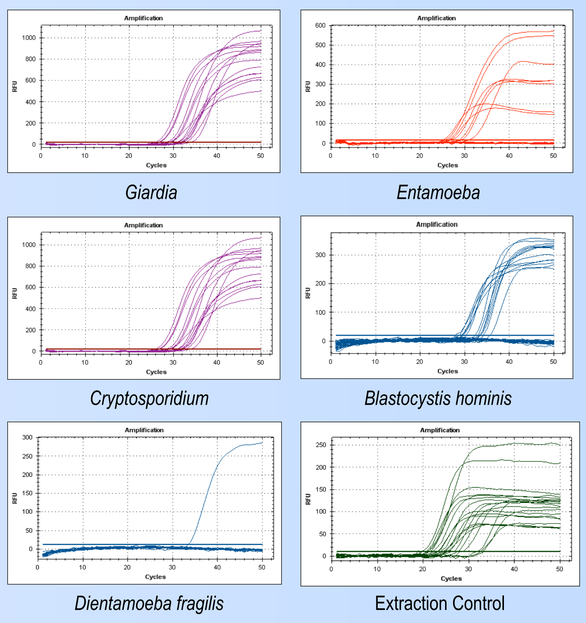


Table 3. Results obtained using the *EasyScreen*™ Parasite assay.

	Faecal Samples (n=81)		Purified DNA (n=50)	
	Microscopy	*EasyScreen*	Previous PCR	*EasyScreen*
Giardia intestinalis	33	37	15	15
Cryptosporidium spp	15	15	15	15
Dientamoeba fragilis	12	13	12	9
Entamoeba complex	N/A	7	8	8
Entamoeba histolytica	0	0	2	2
Blastocystis hominis	15	20*	0	1
No Pathogen Identified	11	6	N/A	N/A
Mixed Infections	7	23	N/A	5

Using the *EasyScreen*™ GI panels we consistently achieved better results that conventional techniques such as culture, microscopy and EIA. The *EasyScreen*™ *C.difficile* detection kit demonstrated 100% concordance when tested against the "Gold-Standard" of Culture Toxin (see Table 2). In addition the method also showed improved performance when tested against two independent molecular assays. The *EasyScreen*™ Parasite assay also demonstrate improved detection when compared to Microscopy and EIA (see Table 3).

Figure 3. Amplification plots obtained using the *EasyScreen*™ Viral assay.

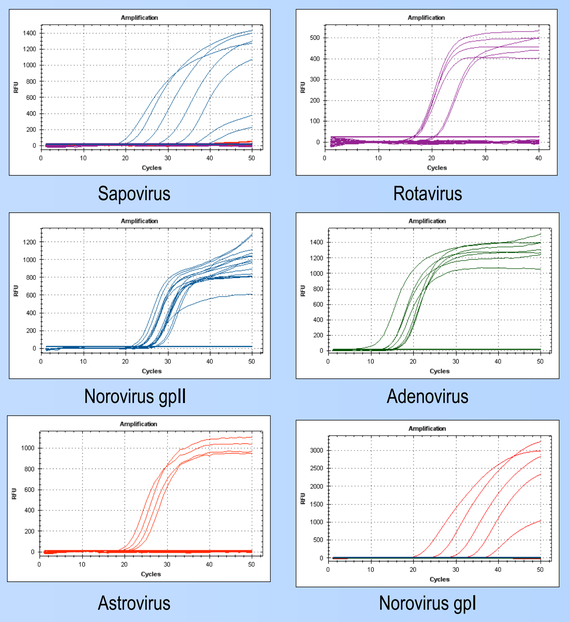


Table 4. Results obtained using the *EasyScreen*™ Viral assay.

	EIA positive	*EasyScreen* Viral positive
Norovirus	81	81 (All group II)
Rotavirus	*21	17
Adenovirus	2	2
Astrovirus	5	6
Sapovirus	N/A	0

*The Rotavirus EIA cross reacts with the vaccine strain

Table 5. Results obtained using the *EasyScreen*™ Bacterial assay.

	Culture	*EasyScreen*
Salmonella	32	31
Campylobacter	40	41
Shigella	1	1
Clostridium difficile	4	4
Yersinia entercolitica	0	0
Listeria monocytogenes	0	0
No Pathogen Identified	3	2

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.

Table 1. Targets detected by the EasyScreen™ GI Panels.

Product #	Description	Microorganisms Detected
CDD001	*EasyScreen*™ C. difficile Detection Kit	Toxigeneic *C. difficile* (targets both *tcdA* and *tcdB*)
CDD002	*EasyScreen*™ C. difficile Reflex Kit	Hypervirulent *C. difficile* incl. 027 & 078 (Targets (i) *tcdC* gene deletion at position 117 (ii) binary toxin gene (iii) *gyrA* gene mutation (fluroquinolone resistance))
EB001	*EasyScreen*™ Enteric Bacteria Detection Kit	(i) *Salmonella spp.* (ii) *Campylobacter spp.* (iii) *Shigella spp.* (iv) *Yersinia sp.*, (v) *toxigenic C. difficile* (vi) *Listeria monocytogenes*
EP001	*EasyScreen*™ Enteric Parasite Detection Kit	(i) *Cryptosporidium spp.*, (ii) *Giardia intestinalis*, (iii) *Dientamoeba fragilis*, (iv) *Entamoeba* Complex and (v) *Blastocystis hominis*
EV001	*EasyScreen*™ Enteric Viral Detection Kit	i) Norovirus I, (ii) Norovirus II, (iii) Astrovirus, (iv) Rotavirus, (v) Sapovirus and (vi) Adenovirus
EP002	*EasyScreen*™ Enteric Microsporidia Detection Kit*	(i) *Enterocytozoon bieneusi* (ii) *Encephalitozoon intestinalis*
EC001	*EasyScreen*™ Pathogenic E. coli Detection Kit*	(i) Enterohaemorrhagic *E. coli* (including O104 strain) (ii) Enterotoxigenic *E. coli* (iii) Enteroinvasive *E. coli* (iv) Enteropathogenic *E. coli* (v) Enteroaggregative *E. coli*

*Coming soon

References

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4. **Baleriola C, Millar D, Melki J, Coulston N, Altman P, Rismanto N and Rawlinson W.** 2008. Comparison of a novel HPV test with the Hybrid Capture II (hcII) and a reference PCR method shows high specificity and positive predictive value for 13 high-risk human papillomavirus infections. J. Clin. Virol. **42**:22-6.
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