

## Supplementary Files

**Table S1.** LiquidArray® Gastrointestinal VER 1.0 panel composition.

PCR1 Pathogens	PCR2 Pathogens
Bacteria	Bacteria
<i>Campylobacter</i> spp.	EAEC
<i>Clostridioides difficile</i> toxins A	ETEC
<i>Clostridioides difficile</i> toxins B	<i>Plesiomonas shigelloides</i>
<i>E. coli eae</i> gene (found in EPEC and EHEC)	<i>Vibrio cholerae</i>
<i>E. coli</i> O157 gene	<i>Vibrio</i> spp.
EIEC/ <i>Shigella</i> spp.	
<i>Salmonella</i> spp.	
Shiga toxin genes <i>stx1/stx2</i> (found in STEC and EHEC)	
<i>Yersinia enterocolitica</i>	
Viruses	Viruses
Norovirus genogroup I (GI)	Adenovirus 40/41
Norovirus genogroup II (GII)	Astrovirus
Rotavirus	Sapovirus
Parasites	Parasites
<i>Cryptosporidium</i> spp.	<i>Ascaris</i> spp.
<i>Entamoeba histolytica</i>	<i>Cyclospora cayetanensis</i>
<i>Giardia lamblia</i>	

Abbreviations: EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; EHEC, enterohaemorrhagic *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; STEC, Shiga toxin-producing *E. coli*.

**Table S2.** Number of samples with valid results in both the LiquidArray Gastrointestinal and comparator methods included in the clinical performance evaluation.

Pathogen	Number of tests performed	Number of invalid LiquidArray tests after repeat	Number of invalid comparator tests after repeat	Number of analysed results <sup>1</sup>
PCR 1				
<i>Campylobacter</i> spp.	1512	0	10	1502
<i>Clostridioides difficile</i> toxin A <sup>2</sup>	1510	0	13	1497
<i>Clostridioides difficile</i> toxin B <sup>2</sup>	1510	0	13	1497
<i>E. coli eae</i> gene	1512	0	5	1507
<i>E. coli</i> O157 gene	1512	0	5	1507
EIEC/ <i>Shigella</i> spp.	1512	0	10	1502
<i>Salmonella</i> spp.	1512	0	10	1502
Shiga toxin genes <i>stx1/stx2</i>	1512	0	5	1507
<i>Yersinia enterocolitica</i>	1512	0	10	1502
Norovirus GI	1512	0	12	1500
Norovirus GII	1512	0	12	1500
Rotavirus	1512	0	12	1500
<i>Cryptosporidium</i> spp.	1512	0	5	1507
<i>Entamoeba histolytica</i>	1512	0	5	1507
<i>Giardia lamblia</i>	1512	0	5	1507
PCR 2		0		
EAEC	1512	0	5	1507
ETEC	1512	0	5	1507
<i>Plesiomonas shigelloides</i> <sup>3</sup>	283	0	0	283
<i>Vibrio cholerae</i> <sup>3</sup>	283	0	0	283
<i>Vibrio</i> spp.	1512	0	10	1502
Adenovirus 40/41	1512	0	12	1500
Astrovirus	1512	0	12	1500
Sapovirus	1512	0	12	1500
<i>Ascaris</i> spp. <sup>3</sup>	110	0	0	110
<i>Cyclospora cayetanensis</i>	1512	0	5	1507

<sup>1</sup> Number of results valid for both LiquidArray Gastrointestinal and comparator methods that were included in the performance analysis. After repeat, none of the LiquidArray Gastrointestinal results were invalid, as opposed to comparator results, resulting in the indicated number of analysed paired valid results. <sup>2</sup> For *C. difficile* toxins A and B testing, 2 out of 1512 comparator data were missing ( $n = 1510$  analysed paired samples; Figure 1). <sup>3</sup> For the very rare pathogens *P. shigelloides*, *V. cholerae* and *Ascaris* spp., only subsets of samples were tested with comparator methods, as described in the Materials and Methods section ( $n = 283$  for *P. shigelloides* and *V. cholerae*,  $n = 110$  for *Ascaris* spp.; Figure 1).

**Table S3.** Indeterminate rates of the LiquidArray Gastrointestinal results among valid paired test results.

Pathogen	Number of analysed results <sup>1</sup>	Indeterminate LiquidArray Gastrointestinal	
		Initial n/N (%)	After repeat n/N (%)
PCR 1			
<i>Campylobacter</i> spp.	1502	2/1502 (0.1%)	0/1502 (0.0%)
<i>Clostridioides difficile</i> toxin A <sup>2</sup>	1497	1/1497 (0.1%)	0/1497 (0.0%)
<i>Clostridioides difficile</i> toxin B <sup>2</sup>	1497	0/1497 (0.0%)	0/1497 (0.0%)
<i>E. coli eae</i> gene	1507	16/1507 (1.1%)	1/1507 (0.1%)
<i>E. coli</i> O157 gene	1507	0/1507 (0.0%)	0/1507 (0.0%)
EIEC/ <i>Shigella</i> spp.	1502	0/1502 (0.0%)	0/1502 (0.0%)
<i>Salmonella</i> spp.	1502	0/1502 (0.0%)	0/1502 (0.0%)
Shiga toxin genes <i>stx1/stx2</i>	1507	0/1507 (0.0%)	0/1507 (0.0%)
<i>Yersinia enterocolitica</i>	1502	0/1502 (0.0%)	0/1502 (0.0%)
Norovirus GI	1500	0/1500 (0.0%)	0/1500 (0.0%)
Norovirus GII	1500	0/1500 (0.0%)	0/1500 (0.0%)
Rotavirus	1500	4/1500 (0.3%)	1/1500 (0.1%)
<i>Cryptosporidium</i> spp.	1507	0/1507 (0.0%)	0/1507 (0.0%)
<i>Entamoeba histolytica</i>	1507	0/1507 (0.0%)	0/1507 (0.0%)
<i>Giardia lamblia</i>	1507	0/1507 (0.0%)	0/1507 (0.0%)
PCR 2			
EAEC	1507	0/1507 (0.0%)	0/1507 (0.0%)
ETEC	1507	2/1507 (0.1%)	0/1507 (0.0%)
<i>Plesiomonas shigelloides</i> <sup>3</sup>	283	0/283 (0.0%)	0/283 (0.0%)
<i>Vibrio cholerae</i> <sup>3</sup>	283	0/283 (0.0%)	0/283 (0.0%)
<i>Vibrio</i> spp.	1502	2/1502 (0.1%)	0/1502 (0.0%)
Adenovirus 40/41	1500	2/1500 (0.1%)	0/1500 (0.0%)
Astrovirus	1500	0/1500 (0.0%)	0/1500 (0.0%)
Sapovirus	1500	1/1500 (0.1%)	0/1500 (0.0%)
<i>Ascaris</i> spp. <sup>3</sup>	110	0/110 (0.0%)	0/110 (0.0%)
<i>Cyclospora cayetanensis</i>	1507	0/1507 (0.0%)	0/1507 (0.0%)

<sup>1</sup> Valid for both LiquidArray Gastrointestinal and comparator methods (Table 4 and Table S2). <sup>2</sup> For *C. difficile* toxins A and B testing, 2 out of 1512 comparator data were missing ( $n = 1510$  analysed paired samples; Figure 1). <sup>3</sup> For the rare pathogens *P. shigelloides*, *V. cholerae* and *Ascaris* spp, only subsets of samples were tested with comparator methods, as described in the Materials and Methods section ( $n = 283$  for *P. shigelloides* and *V. cholerae*,  $n = 110$  for *Ascaris* spp.; Figure 1).

**Table S4.** Confirmation of the clinical performance of the LiquidArray Gastrointestinal assay for the detection of rare pathogens (see Table 4) using contrived samples.

Pathogen	Positive Percent Agreement (PPA) $n/N$ <sup>1</sup> (%)	Negative Percent Agreement (NPA) $n/N$ <sup>2</sup> (%)
PCR1		
<i>Entamoeba histolytica</i>	5/5 (100%)	25/25 (100%)
PCR 2		
<i>Plesiomonas shigelloides</i>	5/5 (100%)	25/25 (100%)
<i>Vibrio cholerae</i>	5/5 (100%)	25/25 (100%)
<i>Vibrio</i> spp.	5/5 (100%)	25/25 (100%)
<i>Cyclospora cayetanensis</i>	5/5 (100%)	25/25 (100%)

<sup>1</sup>  $n/N$  is the ratio of the number of samples positive for the respective pathogen in the LiquidArray Gastrointestinal assay to the number of tested contrived (positive) samples. <sup>2</sup>  $n/N$  is the ratio of the number of samples negative for the respective pathogen in the LiquidArray Gastrointestinal assay to the number of tested negative samples.

**Table S5.** Co-amplification testing of the LiquidArray Gastrointestinal assay.

Co-detected pathogens	Co-amplification result <i>n/N</i> <sup>1</sup> (%)
PCR1	
<i>Campylobacter</i> spp. + <i>Salmonella</i> spp.	3/3 (100%)
<i>Campylobacter</i> spp. + EPEC	3/3 (100%)
<i>Clostridioides difficile</i> (A+B) + EPEC	3/3 (100%)
<i>Clostridioides difficile</i> (A+B) + STEC	3/3 (100%)
<i>Clostridioides difficile</i> (A+B) + Norovirus (GI + GII)	3/3 (100%)
<i>Clostridioides difficile</i> (A+B) + Rotavirus	3/3 (100%)
Norovirus GI + Norovirus GII	3/3 (100%)
Norovirus (GI + GII) + Rotavirus	3/3 (100%)
Norovirus (GI + GII) + EPEC	3/3 (100%)
EIEC/ <i>Shigella</i> spp. + EPEC	3/3 (100%)
<i>Giardia lamblia</i> + EPEC	3/3 (100%)
PCR 2	
Adenovirus + Sapovirus	3/3 (100%)
Astrovirus + Sapovirus	3/3 (100%)

<sup>1</sup> *n/N* is the ratio of the number of samples positive for the respective co-detected pathogens in the LiquidArray Gastrointestinal assay to the number of tested positive samples (either cultured or clinical material combined prior to nucleic acids extraction).

**Table S6.** Cross-reactivity testing of the LiquidArray Gastrointestinal assay in duplicate PCR1 and PCR2 reactions using 59 commensal and non-target pathogens.

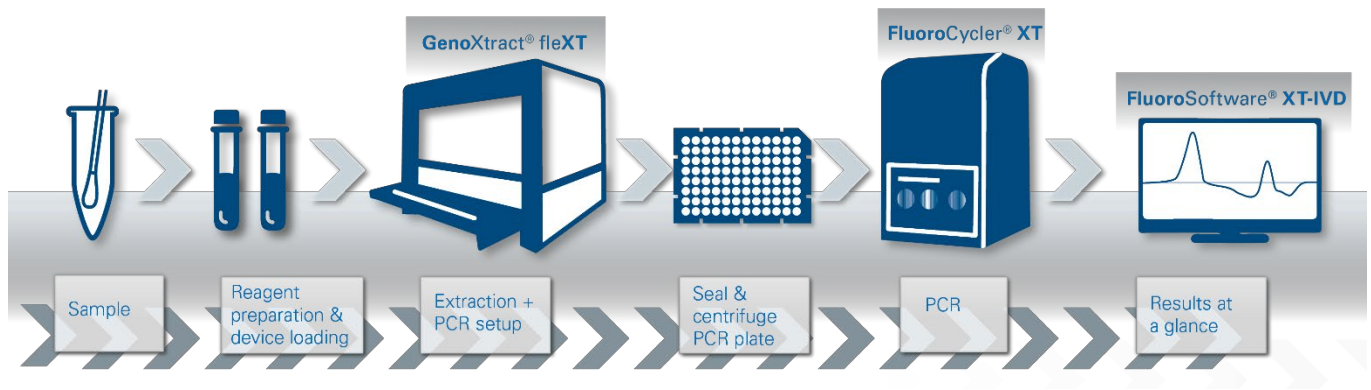
Species	Role	Source / Number	Form tested	Concentration tested
<b>Bacteria</b>				
<i>Abiotrophia defectiva</i>	Normal gut flora	CCUG 27807	Genomic DNA	6.8E+04 ge/rxn
<i>Acinetobacter baumannii</i>	Normal gut flora	DSM 30007	Microorganism	6.5E+05 CFU/ mL
<i>Aeromonas hydrophila</i>	Travellers' diarrhoea	LMG 21108	Genomic DNA	1.0E+07 ge/rxn
<i>Alcaligenes faecalis</i>	Normal gut flora	DSM 6174	Genomic DNA	9.0E+06 ge/rxn
<i>Alistipes finegoldii</i>	Normal gut flora	DSM 17242	Genomic DNA	4.1E+07 ge/rxn
<i>Akkermansia muciniphila</i>	Normal gut flora	DSM 22959	Genomic DNA	1.1E+07 ge/rxn
<i>Arcobacter butzleri</i>	Can cause diarrhoea	DSM 7301	Genomic DNA	1.0E+07 ge/rxn
<i>Bacillus cereus</i>	Can cause diarrhoea	DSM 31	Genomic DNA	3.8E+05 ge/rxn
<i>Bacteroides fragilis</i>	Normal gut flora	DSM 2151	Genomic DNA	1.0E+07 ge/rxn
<i>Barnesiella intestinihominis</i>	Normal gut flora	DSM 21032	Genomic DNA	2.3E+06 ge/rxn
<i>Bifidobacterium bifidum</i>	Normal gut flora	DSM 20215	Genomic DNA	2.0E+06 ge/rxn
<i>Campylobacter fetus</i>	Cross-reactivity	DSM 5361	Genomic DNA	4.2E+05 ge/rxn
<i>Campylobacter concisus</i>	Cross-reactivity	ATCC-BAA-1457D-5	Genomic DNA	5.2E+06 ge/rxn
<i>Citrobacter amalonaticus</i>	Normal gut flora	ATCC25405-D	Genomic DNA	1.6E+06 ge/rxn
<i>Citrobacter koseri</i>	Normal gut flora	ATCC-BAA-895D-5	Genomic DNA	6.3E+04 ge/rxn
<i>Clostridium perfringens</i>	Normal gut flora	DSM 2943	Genomic DNA	2.0E+05 ge/rxn
<i>Clostridium septicum</i>	Normal gut flora	DSM 7534	Genomic DNA	1.2E+05 ge/rxn
<i>Clostridium tetani</i>	Normal gut flora	ATCC19406-D3	Genomic DNA	2.7E+05 ge/rxn
<i>Collinsella aerofaciens</i>	Normal gut flora	DSM 3979	Genomic DNA	2.4E+05 ge/rxn
<i>Edwardsiella tarda</i>	Fish pathogen	ATCC15947-D3	Genomic DNA	1.7E+06 ge/rxn
<i>Eggerthella lenta</i>	Normal gut flora	DSM 15644	Genomic DNA	1.1E+07 ge/rxn
<i>Enterobacter cloacae</i>	Normal gut flora	DSM 30054	Microorganism	1.0E+05 CFU/mL
<i>Enterococcus faecalis</i>	Normal gut flora	DSM 2570	Microorganism	5.0E+05 CFU/mL
<i>Escherichia coli</i>	Normal gut flora	NCTC13476	Microorganism	1.0E+05 CFU/mL
<i>Escherichia coli</i>	Atypical EAEC	DSM 10974	Microorganism	3.7E+06 CFU/ mL
<i>Faecalibacterium prausnitzii</i>	Normal gut flora	ATCC-27766DQ	Genomic DNA	2.0E+05 ge/rxn
<i>Fusobacterium varium</i>	Ulcerative colitis	CCUG 35114	Genomic DNA	2.9E+07 ge/rxn
<i>Gemella morbillorum</i>	Normal gut flora	DSM 20572	Genomic DNA	6.0E+06 ge/rxn
<i>Hafnia alvei</i>	Normal gut flora	DSM 30163	Genomic DNA	6.8E+06 ge/rxn
<i>Helicobacter pylori</i>	Normal gut flora	ATCC700392D-5	Genomic DNA	2.0E+06 ge/rxn
<i>Klebsiella pneumoniae</i>	Normal gut flora	NCTC13442	Microorganism	1.0E+05 CFU/mL
<i>Lactobacillus reuteri</i>	Normal gut flora	DSM 20016	Genomic DNA	5.0E+06 ge/rxn
<i>Listeria monocytogenes</i>	Normal gut flora / foodborne pathogen	DSM 19094	Genomic DNA	3.1E+07 ge/rxn
<i>Morganella morganii</i>	Normal gut flora	DSM 30164	Genomic DNA	4.0E+06 ge/rxn
<i>Parabacteroides distasonis</i>	Normal gut flora	DSM 20701	Genomic DNA	4.1E+06 ge/rxn
<i>Peptoniphilus asaccharolyticus</i>	Normal gut flora	DSM 3032	Genomic DNA	5.4E+05 ge/rxn
<i>Photobacterium damsela</i>	Cross-reactivity	DSM 7482	Genomic DNA	7.7E+05 ge/rxn
<i>Prevotella melaninogenica</i>	Normal gut flora	DSM 7089	Genomic DNA	5.0E+05 ge/rxn
<i>Proteus mirabilis</i>	Normal gut flora	DSM 4479	Genomic DNA	2.0E+06 ge/rxn
<i>Providencia alcalifaciens</i>	Can cause diarrhoea	DSM 30120	Genomic DNA	8.4E+06 ge/rxn
<i>Pseudomonas aeruginosa</i>	Normal gut flora	NCTC13437	Microorganism	1.1E+06 CFU/ mL
<i>Staphylococcus aureus</i>	Normal gut flora	DSM 20231	Microorganism	5.0E+05 CFU/ mL
<i>Streptococcus agalactiae</i>	Normal gut flora	DSM 2134	Genomic DNA	1.2E+06 ge/rxn
<i>Veillonella parvula</i>	Normal gut flora	CCUG 59474	Genomic DNA	5.4E+06 ge/rxn
<i>Yersinia intermedia</i>	Normal gut flora	DSM 18517	Genomic DNA	9.0E+06 ge/rxn
<i>Yersinia pseudotuberculosis</i>	Cross-reactivity	ATCC6904D-5	Genomic DNA	3.2E+06 ge/rxn
<b>Viruses</b>				
Adenovirus E (4)	Respiratory	0810070CFHI	Microorganism	1.1E+05 TCID <sub>50</sub> / mL
Coxsackievirus B3	Gastroenteritis	0810074CFHI	Microorganism	2.0E+06 TCID <sub>50</sub> / mL

Enterovirus 68	Respiratory	0810237CFHI	Microorganism	2.0E+03 TCID <sub>50</sub> / mL
<b>Parasites</b>				
<i>Blastocystis hominis</i>	Normal gut flora	ATCC-50177D	Genomic DNA	9.0E+05 ge/rxn
<i>Dientamoeba fragilis</i>	Normal gut flora	ATCC-PRA-3007SD	Genomic DNA	3.7E+05 ge/rxn
<b>Fungi</b>				
<i>Candida albicans</i>	Normal gut flora	ATCC-MYA-685	Genomic DNA	1.2E+04 ge/rxn
<i>Candida parapsilosis</i>	Normal gut flora	ATCC-22019	Genomic DNA	5.9E+04 ge/rxn
<i>Candida tropicalis</i>	Normal gut flora	ATCC-750	Genomic DNA	2.5E+04 ge/rxn
<i>Candida glabrata</i>	Normal gut flora	ATCC 2001	Genomic DNA	3.4E+05 ge/rxn
<i>Candida krusei</i>	Normal gut flora	ATCC 14243	Genomic DNA	2.7E+04 ge/rxn
<i>Candida lusitaniae</i>	Normal gut flora	ATCC 34449	Genomic DNA	2.6E+05 ge/rxn
<i>Aspergillus versicolor</i>	Environmental origin	DSM 1943	Genomic DNA	3.4E+03 ge/rxn
<i>Saccharomyces cerevisiae</i>	Foodborne	ATCC-18824	Genomic DNA	2.0E+04 ge/rxn

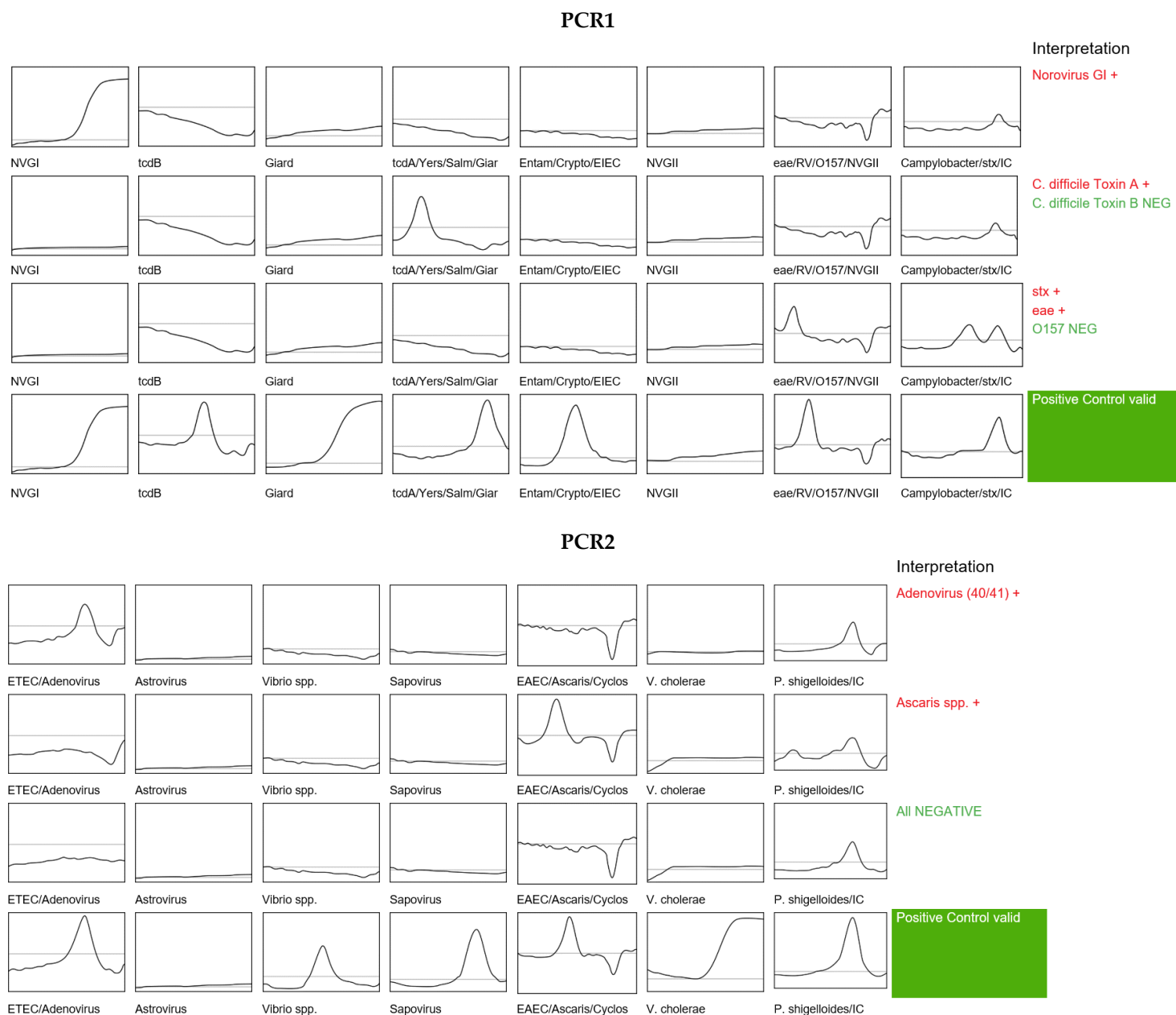
**Table S7.** Precision testing of the LiquidArray Gastrointestinal assay.

Reproducibility		
	Variance (%)	P-value <sup>2</sup>
PCR 1 ( <i>n</i> = 4928) <sup>1</sup>		
Between-operator precision	0.01	0.390
Between-cycler precision	0.01	0.365
Between-run (plate) precision	0.36	0.314
PCR 2 ( <i>n</i> = 3620) <sup>1</sup>		
Between-operator precision	0.00	0.612
Between-cycler precision	0.04	0.074
Between-run (plate) precision	0.66	0.007
Repeatability		
PCR 1 ( <i>n</i> = 4928) <sup>1</sup>		
Probability to get the same result, % [95% CI]	96.9% [96.4–97.4]	
PCR 2 ( <i>n</i> = 3626) <sup>1</sup>		
Probability to get the same result, % [95% CI]	96.2% [95.5–96.8]	

<sup>1</sup> Number of measurements included in precision calculation. <sup>2</sup> ANOVA analysis of variance in combination with a linear mixed effects model. Abbreviation: CI, confidence interval.



**Figure S1.** LiquidArray® Gastrointestinal VER 1.0 workflow. Stool samples are added to Stool Buffer and nucleic acids are extracted with the GXT96 X3 Extraction Kit on the GenoXtract® fleXT automated instrument. Real-time PCR and/or melting curves are generated on the FluoroCycler® XT thermocycler. Acquired fluorescence signatures are interpreted with the FluoroSoftware® XT-IVD software. Results are displayed automatically in the FluoroCycler® Report. LiquidArray® Gastrointestinal VER 1.0 allows the analysis and interpretation of 48 stool samples in about 5 hours.



**Figure S2.** LiquidArray® Gastrointestinal VER 1.0 result reporting. Examples of fluorescence signatures for PCR1 and PCR2 testing, and their interpretation (positive/negative).