External Quality Assessment panel for the Detection of Gastroenteritis viruses

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INTRODUCTION

Gastroenteritis is a common condition resulting from a viral, bacterial or parasitic infection which can manifest as outbreaks. Viral gastroenteritis is mostly transmitted by faecal-oral route, direct contact with an infected person or by contaminated food and water. The most common symptoms of gastroenteritis are diarrhoea, nausea, vomiting, lack of appetite and fever.

The number of confirmed sporadic cases and outbreaks due to norovirus continue to rise in the UK and worldwide. Winter season is the peak season for outbreaks hence the name 'winter vomiting bug'[1]. Rotavirus affects mostly young children up to the age of 5 and was the most frequent cause of hospitalisation of children with diarrhoea in the UK[2]. Adenovirus types 40 and 41 are responsible for a smaller number of cases in adults and children.

Viral particles can be identified by immunoelectron microscopy however molecular and immunological methods are used for diagnosis because they are more adapted for routine use[3]. The availability of an External Quality Assessment (EQA) panel for the detection of enteric viruses is crucial for providing objective evidence of the quality of testing. This poster summarises the results obtained with pilot studies which were performed in the development of an EQA scheme aimed at the molecular and antigen detection of gastroenteritis viruses.

MATERIAL & METHODS

Screening of Stools:
Anonymised stool samples from patients with symptoms of acute gastroenteritis, tested positive for the presence of Adenovirus, Norovirus genogroup I/II and Rotavirus, were collected to prepare positive specimens for distribution. All stool samples used in the pilot studies were screened negative for *Escherichia coli* O157, salmonella and shigella prior to use.

Enzyme Immunosorbent assay (EIA) were used in house to screen all specimens: Oxoid IDEA™, ProSpecT Adenovirus and ProSpecT Rotavirus. RIDA®QUICK Norovirus and VIKIA® Rota-Adeno were used for rapid testing and an in house real-time PCR assay was used to determine the Cycle threshold (Ct) values.

Specimen preparation and design:
The stool samples were selected based on their Ct value and EIA index results. Where required samples that had similar Ct values were pooled together to provide sufficient volume for distribution. Each specimen consisted of 1mL of freeze-dried stool suspension in a 0.3% Bovine Serum Albumin (BSA) matrix.

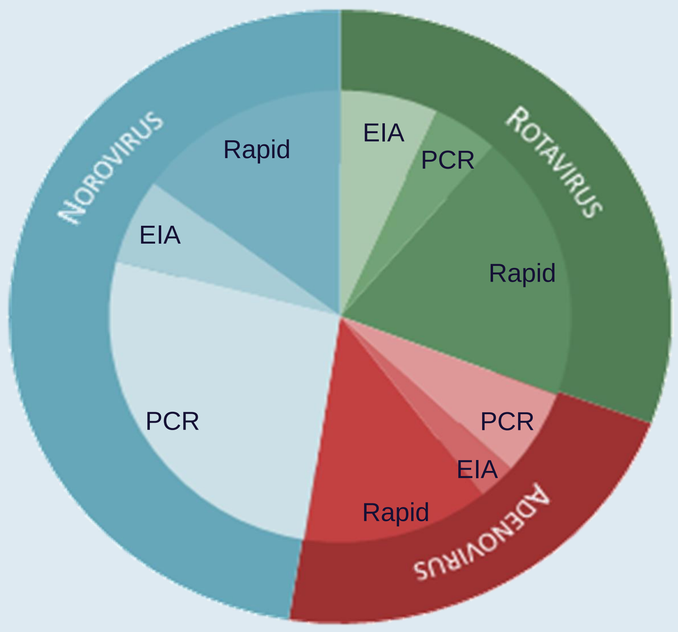
RESULTS

Table 1: Summary of the specimen details and the performance of the different assays used during pilot studies

	Intended Result	Adenovirus Positive			Norovirus Positive						Rotavirus Positive			Rotavirus PCR Positive, Ag Negative	Rotavirus Positive	
	Type	AD41	AD41	AD41	GII-4	GII-4	GII-4	GII-4	GII	GII	G1P8, subtype C	G1P8	RC3	RC3	R18	R15
	Specimen number	2483	2484	3058	1928	1929	1930	1931	2485	3061	2481	2482	3059	3060	3062	3063
	Content	Negative stools spiked with culture stain	1:10 dilution of specimen 2483	1:5 dilution of cultured strain	Pool of positive stools	Pool of positive stools	Pool of positive stools	1:10 of specimen 1930	Pool of positive stools	Pool of positive stools	Pool of positive stools	Positive stools spiked with culture stain	1:5 Dilution from cultured strain	1:70 Dilution from cultured strain	1:28 Dilution from clinical specimen	1:85 Dilution from clinical specimen
	in house PCR (cut-off Ct > 40)	19.2	21.0	15.0	31.5	27.8	15.0	16.8	21.0	24.6	22.4	26.9	19.7	23.6	14.1	16.2
PCR	In-house PCR	5/5	5/5	5/5	9/11	10/11	11/11	11/11	10/10	9/9	6/6	6/6	6/6	5/6	6/6	6/6
PCR	r-biopharm RIDA®GENE viral stool panel I&II	2/2	2/2	2/2	3/4	4/4	4/4	4/4	5/5	-	1/1	1/1	-	-	-	-
PCR	bioMerieux R-Gene®	1/1	1/1	1/1	-	-	-	-	1/1	4/4	-	-	-	-	-	-
PCR	Cepheid SmartCycler®	-	-	-	4/4	4/4	4/4	4/4	4/4	1/1	-	-	-	-	-	-
PCR	Cepheid GeneXpert®	-	-	-	-	-	-	-	3/3	3/3	-	-	-	-	-	-
PCR	Unspecified	-	-	1/1	-	-	-	-	-	4/4	-	-	1/1	1/1	1/1	1/1
EIA	ProSpecT® Rotavirus	-	-	-	-	-	-	-	-	-	2/3	2/3	2/2	2/2	2/2	2/2
EIA	Oxoid IDEIA™ NOROVIRUS	-	-	-	0/4	1/4	4/4	4/4	-	1/2	-	-	-	-	-	-
EIA	r-biopharm RIDASCREEN®	1/1	0/1	1/1	0/2	1/2	2/2	2/2	3/3	2/2	1/1	1/1	1/1	0/1	1/1	1/1
RAPID	CorisBioconcept Combi-Strip	0/6	0/6	2/6	-	-	-	-	-	-	1/7	1/7	5/6	5/6	6/6	6/6
RAPID	Biorapid Rota/Adeno	2/4	0/4	3/3	-	-	-	-	-	-	0/4	0/4	3/3	3/3	3/3	3/3
RAPID	r-biopharm RIDA®QUICK Norovirus	-	-	-	0/8	1/8	6/8	7/8	7/8	7/8	-	-	-	-	-	-
RAPID	ImmunoCard STAT!®	-	-	-	0/1	0/1	1/1	1/1	1/1	-	1/7	4/7	1/1	1/1	1/1	1/1
RAPID	Rapid Strip ROTA/ADENO	-	-	-	-	-	-	-	-	1/1	-	-	2/5	4/5	5/5	5/5
RAPID	Unspecified	-	-	3/4	-	-	-	-	-	3/3	-	-	5/7	5/7	6/7	7/7
	Overall participants	45	45	43	35	35	35	35	45	43	45	45	43	43	43	43
	PCR assay ,performance	100.0%	100.0%	100.0%	84.20%	94.7%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	90.0%	100.0%	100.0%
	EIA, performance	100.0%	33.3%	75.0%	0.00%	33.3%	100.0%	100.0%	100.0%	66.7%	50.0%	66.7%	100.0%	66.6%	100.0%	100.0%
	Rapid assay, performance	31.6%	0.0%	66.6%	7.70%	15.4%	76.9%	92.3%	92.3%	92.3%	16.0%	32.0%	72.0%	76.0%	96.0%	100.0%

- Assays with three or less users across all specimens have been excluded from the table summary

Figure 1: Proportion of participants using different assays by virus



DISCUSSION OF RESULTS

- For the positive specimens the percentage of correct results correlated with specimen viral load and was influenced by the method used, with the molecular assays being the most sensitive.
- Specimen numbers 1928 and 1929 had the lowest performance in Norovirus testing. These specimens were positive with RT-PCR and negative with EIA in pre-distribution testing.
- Specimen 2481 was a weak positive sample and it had the lowest performance with rapid assays of all Rotavirus samples that were distributed.
- The detection of rotavirus Ag varied with the source material used to prepare specimen 2481 (rotavirus positive pool) and 2482 (negative pool spiked with a cultured strain) and with the different rapid kits used.
- The results of 2483 and 2484 specimens highlighted that the dilution of the original specimen lead to the lack of sensitivity of EIA used for testing. The Ct values greater than 25 obtained that the low viral load for these two specimens.
- Participants performing molecular methods used a variety of extraction amplification platforms and kits which resulted in a variety of Ct values.

CONCLUSIONS

- A pilot studies confirmed that the selected format of freeze-dried stool suspension is suitable for use by both molecular and antigen detection assays.
- Due to the success of the pilot studies an EQA panel called Viral Gastroenteritis has been introduced. The panel is aimed at the detection of adenovirus 40 and 41, norovirus and rotavirus.

KEYWORDS

EQA, Adenovirus, Norovirus, Rotavirus, Viral gastroenteritis, EIA, PCR, Rapid assay

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