Developing an External Quality Assessment scheme for the detection of Gastroenteritis viruses

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1. Introduction

Noroviruses have a significant role in both sporadic and epidemic gastroenteritis worldwide. Globally, norovirus is associated with nearly 50% of all gastroenteritis outbreaks and more than 90% of viral epidemic gastroenteritis[1]. Norovirus cases are reported throughout the year. However most outbreaks peak in the winter season hence the name of 'winter vomiting bug'[2]. Rotavirus remains the leading cause of sporadic gastroenteritis in children under five in countries where vaccination has not been implemented[3]. Although less frequent, Adenovirus types 40 and 41 can cause sporadic gastroenteritis, mostly in adults.

The number of confirmed cases and outbreaks due to norovirus continues to rise in the UK and worldwide. Viral particles can be identified by immunoelectron microscopy however molecular and immunological methods are used for diagnosis because they are more adapted for a routine use[4]. Availability of an External Quality Assessment (EQA) panel for norovirus is crucial for providing objective evidence of the quality of testing.

UK NEQAS (United Kingdom National External Quality Assessment Service) for Microbiology which provides 46 external quality assessment schemes to 1700 laboratories in over 50 countries worldwide, is developing an EQA panel for the detection of gastroenteritis viruses. Feedback from a questionnaire sent by UKNEQAS revealed that a molecular method is the first method of choice (50%) to detect norovirus followed by rapid antigen testing (30%) and EIA (20%).

2. Aims

- To design an EQA specimen format suitable for the detection of norovirus antigen and nucleic acid in faeces.
- To prepare and analyse a pilot distribution to evaluate whether the designed specimens are suitable for external quality assessment
- To further extend the scheme to the detection of rotavirus and adenovirus 40, 41 in the same specimen format

3. Material & Methods

Screening of stools:

Anonymised stool samples from patients with symptoms of gastroenteritis, tested positive for the presence of Norovirus genogroup II, were collected to prepare positive specimens

Anonymised stool samples screened negative for bacteriology, parasitology and virology pathogens were collected to prepare negative specimens.

All stool samples were 1/ screened negative for *Salmonella*, *Escherichia Coli* 0157 and *Shigella; 2/* tested for norovirus with an Enzyme Immunoassay (EIA) kit (Oxoid IDEA™) and with on in house real time assay.

Specimen preparation and design:

Stool samples were selected based on PCR Ct (cycle threshold) values and EIA index results.

Samples with similar ct values/index were pooled to form pilot specimens of sufficient volume for distribution.

A panel of six specimens were prepared, each consisting of 1ml of freeze-dried stool suspension in a 0.3% BSA (Bovine serum albumin) matrix .

Prepared specimens were then tested for norovirus with Oxoid IDEA™ EIA and by an In house real time PCR.

4. Results / specimen format

- A process for producing homogenous and stable EQA specimens suitable for the detection of norovirus Ag and nucleic acid was developed from preliminary studies.
- Stool samples suspended in 0.3% BSA were centrifuged and the supernatant lyophilised.
- Using the supernatant rather than the whole faecal specimen was found to be easier in dispensing and avoid causing blockages in automated plate washers.

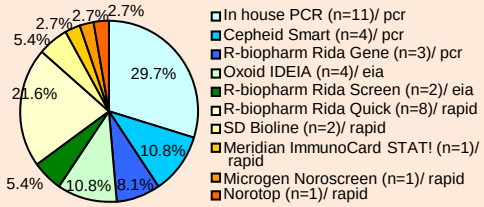
4. Results / pilot distribution

Participation in the pilot:

35 out of 41 participants returned results with one laboratory performing three different assays and one laboratory using two different nucleic acid extraction platforms for the same molecular assay. Altogether 38 sets of results were received.

Assays used to detect Norovirus:

The majority of laboratories used a molecular assay for norovirus detection (48.6%) with in house PCR being the most popular assay. Other methods used were rapid immunoassays (35.2%) and enzyme immunoassays (16.2%).



Pre-distribution and participants results:

Results are presented in table 1 with specimens ordered by decreasing viral load from left to right. Specimens 1928 to 1931 were prepared from three different pools of stools (A, B and C) containing varied amounts of Norovirus genogroup II with specimen 1931 being a 10-fold dilution of specimen 1930.

The percentage of positive correct results correlated with specimen strength and was influenced by the method, with the molecular assays being the most sensitive. The lowest correct performances were obtained for the two specimens with positive PCR results and negative EIA results in pre-distribution testing (1929 & 1930). Ct values greater than 25 obtained confirmed the low viral load in these two specimens. Inhibitory results were reported by one participant for the strongest specimen.

Specimens 1926 and 1927 were negative specimens containing the 0.3% BSA matrix or negative stool respectively. Only one false positive result was reported for specimen 1927 using Oxoid IDEIA.

Table 1 qualitative results by assays and performances by specimen

	Specimen	1930	1931	1929	1928	1926	1927
	Intended	Positive	Positive	Positive	Positive	Negative	Negative
	Content	Pool C	1:10 of 1930	Pool B	Pool A	matrix only	negative stool
	Type	GII-4	GII-4	GII-4	GII-4	-	-
pre-D	In house PCR, Ct Value (40)	15.0	16.8	**27.8**	**31.5**	>40	>40
pre-D	Oxoid IDEIA, index (1)	17	4.6	**0.25**	**0.17**	0.17	0.34
PCR	"In house" PCR, n=11	11	11	10	9	11	11
PCR	R-biopharm Rida Gene, n=4	4	4	4	3	4	4
PCR	Cepheid Smart, n=4	4	4	4	4	4	4
EIA	Oxoid IDEA, n=4	4	4	1	0	4	3
EIA	R-biopharm Rida Screen, n=2	2	2	1	0	2	2
RAPID	R-biopharm Rida Quick, n=8	6	7	1	0	8	8
RAPID	SD Bioline, n=2	2	2	1	1	2	2
RAPID	Microgen Noroscreen, n=1	0	1	0	0	1	1
RAPID	Meridian ImmunoCard STAT!, n=1	1	1	0	0	1	1
RAPID	Norotop, n=1	1	1	0	0	1	1
	All, n=38	35	37	22	17	38	37
	Performance	**92.1%**	**97.4%**	**57.9%**	**44.7%**	**100.0%**	**97.4%**
	PCR assay, performance	100.0%	100.0%	94.7%	84.2%	100.0%	100.0%
	EIA, performance	100.0%	100.0%	33.3%	0.0%	100.0%	83.3%
	Rapid assay, performance	76.9%	92.3%	15.4%	7.7%	100.0%	100.0%

The 19 participants performing a molecular method used a variety of extraction and amplification platforms leading to 19 single combinations. As a consequence Ct values covered a large range of values (table 2).

Positive specimens	*number*	1930	1931	1929	1928
Pre-distribution (n=1)	*Ct value*	15.0	16.8	27.8	31.5
Participants (n=19)	*mean Ct*	19.3	22.2	28.4	31.6
	min Ct	10.7	14.2	21.7	25.7
	max Ct	32.1	26.9	34.3	36.0

Table 2 mean and range of Ct values reported

Genogrouping was carried out by 15 laboratories using in house PCR (n=8), R-biopharm Rida Gene (n=3), Cepheid Smart (n=3) and SD Bioline (n=1); all of which stated the correct genogroup when specimens were detected positive.

5. Conclusions

- The pilot distribution confirmed that the selected format of freeze-dried stool suspension is suitable for molecular and Ag assays. These specimens were shown to be to be stable over the period from preparation to the close of the distribution.
- Participants' results were in agreement with those obtained by our pre distribution tests.
- Overall results highlight that most molecular assays are able to detect small amounts of norovirus that can be present in the stools of symptomatic patients whilst antigen detection assays lack sensitivity to do so.
- A second pilot panel which included specimens positive for norovirus, rotavirus and adenovirus 41 was distributed in March 2014 Results data are currently being analysed. Preliminary results show that 55% of participants used molecular method to detect norovirus whilst 24% and 18.6% used molecular methods to detect rotavirus and adenovirus respectively. Performance was excellent for norovirus detection (92%) and varied from 35% to 63% for the rotavirus and adenovirus. Analysis of results by method is on-going.

References

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