Development of an EQA Scheme for Norovirus Testing

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Aims

- To determine the levels of interest in an EQA scheme for Norovirus testing.
- To determine testing practice for Norovirus detection.
- To prepare a pre-pilot distribution of stool samples for the detection of Norovirus and analyse the results reported.

Introduction

Norovirus is the most common cause of gastroenteritis [1] . It is particularly prevalent in enclosed environments such as hospitals, cruise ships and nursing homes. The virus has a low infectious dose and is spread through the faecal-oral route. Outbreaks of the virus in hospitals cost the NHS an estimated £13 million a year due to ward closures and staff absences. [2]

Rapid identification is important for the implementation of infection control measures. Identification assays are front line tools and the results will influence outbreak investigation. To assess the quality of such assays, UK NEQAS intends to develop an EQA scheme beginning with a pre-pilot distribution for Norovirus identification.

Questionnaire

Method

In March 2011, a questionnaire was sent to all existing UK NEQAS for Microbiology participants asking; if they test for enteric viruses (Norovirus, Rotavirus and Adenovirus 40, 41), which assays are used and if the participant would be interested in an EQA scheme provided by UK NEQAS.

Results

- Out of 243 replies, 161 (66%) laboratories routinely test for at least one enteric virus. (94 UK laboratories).
- 97 (60%) routinely perform a Norovirus detection assay. (44 UK laboratories).
- 77 (79%) would be interested in participating in a UK NEQAS EQA scheme for Norovirus, including 38 UK laboratories (86%).

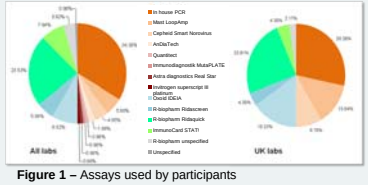

Figure 1 – Assays used by participants

Questionnaire

- The most popular assay format is RT-PCR with 50 labs (52%) reporting its use. (23 (52%) of UK labs); This includes ten multiplex assays.
- 15 (15%) laboratories use an EIA (9 UK)
- 32 (33%) laboratories use a rapid antigen detection assay (13 UK).
- 6 laboratories (6%) use two or more assays (2 (2%) UK).
- 16 laboratories perform Norovirus typing. (9 UK).

Pre-pilot distribution

Method

- 38 UK laboratories were invited to take part in the pilot based on their interest in an EQA scheme in the questionnaire; 22 accepted the invitation.
- Specimens were derived from Norovirus positive faecal material.
- Negative faecal material was also obtained to create negative specimens.
- Semi-solid faecal materials were suspended in 5ml of PBS, 1% Tween-80 to provide sufficient volume.
- Liquid faecal materials were only diluted 1:2 with PBS, 1% Tween-80.
- The Oxoid IDEIA Norovirus was selected to perform pre-distribution testing, with a confirmatory RT-PCR assay being performed by the Enteric Virus Unit, Colindale HPA.
- Pre-distribution results are shown in Table 1 for the six specimens selected.
- It was found that the levels of antigen in the samples were not as high as expected and therefore participants using an EIA or rapid antigen detection kit were asked not to further dilute the samples.
- 200µL of each specimen was distributed to the participants.
- They were given three weeks to perform the testing and return results to UK NEQAS.

Specimen Number	RT-PCR	CT value	Genotype	EIA	Overall result
0828	Pos	24.2	GII	Positive	RNA +ve Ag +ve
0829	Pos	20.0	GII	Positive	RNA +ve Ag +ve
0830	Pos	29.6	GII	Negative	RNA +ve Ag -ve
0831	Neg	-	-	Negative	RNA -ve Ag -ve
0832	Pos	30.9	GI	Negative	RNA +ve Ag -ve
0833	Pos	~17	GII	Positive	RNA +ve Ag +ve

Table 1 – The six specimens sent to participants

Pre-pilot distribution

Results

Out of the 22 laboratories, 20 returned their results.

The majority of laboratories used a molecular assay for Norovirus detection (55%). Other methods used were rapid immunoassays (30%) and enzyme immunoassays (15%). Assays used are summarised in Figure 2.

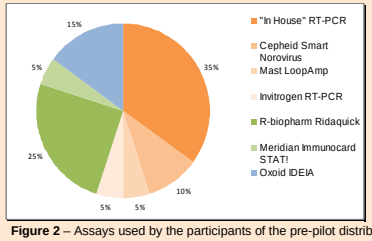

Figure 2 – Assays used by the participants of the pre-pilot distribution

Figure 3 shows the percentage of laboratories correctly detecting Norovirus in the specimens (according to RT-PCR pre-distribution results).

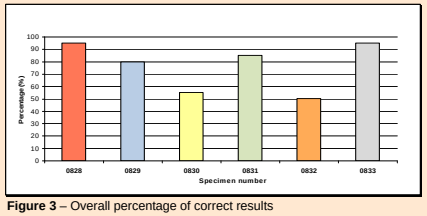

Figure 3 – Overall percentage of correct results

The specimens containing a higher level of Norovirus antigen and RNA, as confirmed by pre-distribution testing, performed the best, alongside the negative specimen. (0828, 0829, 0833 & 0831).

Across all six specimens, the most sensitive method appears to be the molecular method. The enzyme Immunoassays (EIAs) detected only the strongest positive specimens (0828, 0829 & 0833). The rapid immunoassay appears to be the least sensitive detection method (Fig 4).

For the negative specimen (0831), there were two false positives reported. The assays obtaining the false positives were an "In House" RT-PCR and a RIDA®QUICK rapid immunoassay.

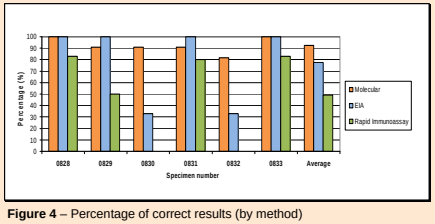

Figure 4 – Percentage of correct results (by method)

Genogrouping was carried out by nine laboratories; all of which stated the correct genogroup for the GII specimens. One laboratory did not detect the GI specimen and another genogrouped the negative specimen.

Participants were asked to test the sample neat if using an EIA or rapid antigen detection kit. It is unclear how many followed this instruction.

The 200µl of faecal material distributed was not adequate for all tests if performed neat; the RIDA®QUICK assay requires 250µl. Many felt that 1ml would be more suitable.

Conclusions

- A larger volume of sample may need to be distributed in the future if the sample is not to be further diluted.
- Dilution of faecal specimens to increase the volume is not ideal as diluted specimens may not be suitable for the less sensitive assays that detect Norovirus antigen. Pooling the same genogroup Norovirus could be considered for future distributions.
- Norovirus antigen may not be as stable as the RNA. Faecal material for specimens needs to be as fresh as possible and may require an antigen stabiliser.
- A stability study is currently on-going; testing for the presence of the antigen in specimens stored at different temperatures, with or without adding a stabiliser. This is to establish the best conditions for faecal material collection and for specimen storage.

Acknowledgements

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References

[1] - Grant S. Hansman *Caliciviruses: Molecular and Cellular Virology*, 2010, Chapter One; *Norovirus Epidemiology*, J. Joukje Siebenga, Erwin Duizer and Marion P.G. Koopman

[2] - *Norovirus outbreaks; time equals money*, Pages134-135, Pathology in Practice, Volume 11, Issue 4, November 2010