BRITISH JOURNAL OF BIOMEDICAL SCIENCE, 2017
VOL. 74, NO. 3, 144–147
https://doi.org/10.1080/09674845.2017.1278886

BIOMEDICAL SCIENCE IN BRIEF

Evaluation of the Xpert Norovirus assay for the rapid detection of norovirus genogroups I and II in faecal specimens within a routine laboratory setting

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Article History Received 28 September 2016; Accepted 6 December 2016

Keywords Norovirus; Xpert norovirus; CerTest norovirus; infection control; PCR; gastroenteritis

Norovirus infection accounts for over 90% of all viral gastroenteritis cases and approximately 50% of all global outbreaks [1]. It is estimated that norovirus infects over 267 million people and may cause 200,000 deaths per year [2]. In the young, elderly, and immunocompromised, the disease may be life threatening due to dehydration but is rarely the sole cause of death [3]. Cases of norovirus may be sporadic or part of outbreaks in closed communal facilities, such as nursing homes, hospitals, creches and cruise ships [4]. According to the Health Protection Surveillance Centre (HPSC), annual norovirus infection rates in Ireland are estimated to be between 1 and 5% of the population [5]. In 2012, there were between 1000 and 1800 notified cases of norovirus and approximately 200 norovirus outbreak notifications in Ireland [6].

Noroviruses are single-stranded RNA, non-enveloped viruses of the genus *Norovirus*, family *Caliciviridae,* which cause acute gastroenteritis in humans and other mammals [7]. Noroviruses can be classified into five different genogroups of which genogroup I (GI) and genogroup II (GII) cause the majority of the infections in humans [8]. Since 2002, a single genogroup, GII.4 has been the dominant norovirus strain detected globally and in Ireland [9]. Norovirus transmits via the faecal–oral route, via aerosolisation of viral particles in vomitus [4] and contamination of food and water [10]. Outbreaks of norovirus cause significant health and cost implications and can be difficult to control [11]. A study in Avon, England, postulated that due to lost bed-days and staff absence, the cost impact of norovirus upon the hospital was estimated to be £635,000 per 1000 beds [12]. Extrapolating this data showed that gastrointestinal outbreaks involving norovirus were likely to cost the English NHS £115 million from April 2002 to March 2003 [12]. The rapid identification of norovirus has important implications in infection prevention and control measures and may reduce the need for additional diagnostic testing [13].

Faeces is the specimen of choice for norovirus testing because the yield of the virus is higher than in other specimens such as vomitus [13]. Electron microscopy was the original diagnostic method used to visualise norovirus in faecal samples directly but has poor sensitivity (~17%) [13]. Commercially available rapid immunochromatographic assays (RIA) have proven to be useful during norovirus outbreak situations but due to low assay sensitivity, RIA's are helpful only when the prevalence of norovirus infection is high [1]. Currently, available RIA's are known to have low sensitivity (17–83%) and moderate to excellent specificity (87–100%) [1,14]. Real-time reverse transcription-polymerase chain reaction (RT-PCR) is the gold standard for the definitive detection and typing of norovirus [1]. Numerous real-time norovirus RT-PCR assays, both in-house and commercial have been developed over the years to detect norovirus in faeces [1,15]. The Xpert Norovirus assay is an automated, qualitative real-time multiplex RT-PCR assay performed on the Cepheid GeneXpert platform. The assay uses primers and probes to detect and amplify unique gene sequences within a conserved region of the norovirus genome allowing the identification and genogroup differentiation of norovirus G1 and GII from raw or unpreserved unformed stool specimens.

In this study, the laboratory performance characteristics of Xpert Norovirus assay were evaluated against a molecular reference method, supplied by the National Virus Reference Laboratory (NVRL) in Dublin, Ireland. Additionally, the Xpert Norovirus assay was compared against the CerTest Norovirus assay, a qualitative immune-chromatographic assay responsible for the simultaneous detection of Norovirus GI and GII in faecal specimens.

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The study tested both fresh (collected from September 2014 to January 2015) and frozen faecal specimens (collected from October 2013 to November 2014). Faecal samples for this study were obtained from the Midland Regional Hospital Tullamore (MRHT) and the NVRL. Samples from both sites were anonymised before testing began. The sample size was chosen on the basis of the associated confidence interval. With a low expectation of failure rates, a sample of 100 and an observed failure rate of zero, the 97.5% upper Poisson confidence interval is 3.5%. With a failure rate as high as 5%, the confidence interval is 1.6 to 11.2%, a width of roughly ±5% around the estimate, which was deemed an acceptable margin of error in the estimation. The 104 faecal specimens used in the study originated from patients in hospitals and nursing homes that presented with acute gastroenteritis. Of the 104 stool samples, 80 frozen stool specimens were obtained from the NVRL. These specimens were previously tested fresh by the NVRL's reference PCR method and subsequently frozen. All frozen samples obtained from the NVRL by MRHT were frozen on receipt and later kept at –80 °C until testing. The remaining 24 fresh faecal specimens originated from MRHT and were tested by Xpert Norovirus (Cepheid, Sunnyvale, CA, USA) and CerTest Norovirus (CerTest, Biotec, Spain) within 24 h of collection. Simultaneously, an aliquot (>1 g or 1 ml) from each fresh faecal specimen was immediately sent to the NVRL at a temperature of 2–8 °C. Fresh faecal specimens sent to the NVRL were tested by NVRL's reference PCR assay within 24 h of dispatch from MRHT.

The Xpert Norovirus assay was performed as per manufacturer's instructions. Referring to manufacturer diagrammatic specifications an appropriate amount of stool specimen was transferred to a sample reagent bottle (containing lysis buffer solution) using a sterile swab. The inoculated reagent bottle was vortexed at 3000 rpm for 10 s. The solution was then transferred into the sample port of the Xpert Norovirus cartridge using a sterile pipette and loaded onto the GeneXpert DX system. The test was repeated if the result was 'invalid', 'error', or 'no result'.

Upon receipt into the NVRL, approximately 20% w/v of the faecal sample was suspended in 400 µl S.T.A.R buffer (Roche) The samples were externally lysed and extracted by a Roche MagNAPure 96 as per manufacturer protocol. Eluates were tested on an internally controlled multiplex one-step real-time RT-PCR for norovirus detection and genotyping as previously described [16] with modification of internal control [17]. Eluates were tested in a 25 µl reaction mixture containing 2× Superscript™ III Platinum One-Step qRT-PCR mix, as per product insert. Final concentrations of norovirus primers and probe were 400 and 80 nM respectively, and BMV primers and probe were 200 nM and 100 mM respectively. Amplification was performed on the ABI 7500 Fast instrument under the following conditions; 15 min 50 °C, 2 min 95 °C, 38 cycles of 15 s 95 and 30 s 56 °C. Amplification data was collected and analysed with Sequence Detection Software version 2.3 (Applied Biosystems).

The CerTest Norovirus GI + GII assay was performed as per manufacturer's instructions. 125 mg of stool specimen (or 125 µl for liquid stool specimens) was transferred to a stool collection tube and mixed thoroughly. An appropriate amount of sample was then applied to the CerTest Norovirus card. Results were read after 10 min and interpreted using manufacturer instructions. Invalid results were repeated.

The 104 faecal specimens were each tested for Norovirus by the Xpert Norovirus, NVRL Reference PCR and CerTest Norovirus assays. The norovirus status was defined by the results from the NVRL assay. The panel consisted of eight norovirus GI, 41 norovirus GII and 55 negative samples for norovirus RNA. Of the 104 specimens tested, there was an Xpert Norovirus internal quality control (IQC) invalid rate of 2.9% (3/104) of samples on initial testing. All three initial invalid results subsequently passed IQC when repeated resulting in a valid assay result. The CerTest Norovirus and NVRL Reference PCR assays had no IQC invalid/failures for all 104 specimens tested (0/104). Table 1 shows assay performance (sensitivity, specificity, PPV and NPV) of Xpert Norovirus and CerTest Norovirus.

Molecular genetic testing for norovirus infection plays a significant role in patient management and can reduce healthcare-associated costs in outbreak situations by providing accurate and timely results [11]. Rapid detection of norovirus allows health care providers to limit the use of unnecessary antimicrobial agents, assign isolation resources more efficiently and potentially decrease the length of stay in a hospital setting [11]. Xpert Norovirus displayed excellent performance characteristics when compared to NVRL's RT-PCR reference method for all samples tested (Table 1). Two recent studies [15,18] evaluated Xpert Norovirus using a laboratory-developed RT-PCR as a reference method. Data from both studies were compared well with data from this study. Rovida et al. reported similar performance characteristics to those shown in this study by having a 95.4% correlation with the reference method. Gonzalez et al. reported Negative predictive value (NPV) and Positive predictive value (PPV) of 75 and 100% for norovirus GI and 99.9 and 86.5% for norovirus GII, respectively. No study has reported a direct method comparison of Xpert Norovirus with other commercially available molecular assays. However, a FilmArray GI Panel evaluation using an RT-PCR reference method to detect norovirus in faeces reported sensitivity and specificity of 94.5 and 98.8%, respectively [19]. These values are similar to those shown by Xpert Norovirus in this study. Invalid results obtained in this study were likely the cause of operator error whereby an excess or deficit of sample was applied to a sterile swab during sample preparation. In MRHT, the risk

Table 1. Summary of Assay performance results.[1]

	Number of[2]				Sensitivity%	Specificity%	PPV%	NPV%
Assay/Number of Samples	TP	FP	FN	TN		Norovirus Genogroup Combined		
Xpert Norovirus ($n = 104$)	49	0	0	55	100	100	100	100
Frozen ($n = 80$)	36	0	0	44	100	100	100	100
Fresh ($n = 24$)	13	0	0	11	100	100	100	100
CerTest Norovirus ($n = 104$)	36	2	13	53	73.5	96.4	94.7	80.3
Frozen ($n = 80$)	26	2	11	41	70.3	95.4	92.9	78.9
Fresh ($n = 24$)	10	0	2	12	83.3	100	100	85.7
						Norovirus Genogroup I		
Xpert Norovirus ($n = 104$)	8	0	0	96	100	100	100	100
Frozen ($n = 80$)	8	0	0	72	100	100	100	100
Fresh ($n = 24$)	0	0	0	24	–	100	–	100
CerTest Norovirus ($n = 104$)	5	1	3	95	62.5	99.0	83.3	96.9
Frozen ($n = 80$)	5	1	3	71	62.5	98.6	83.3	96.0
Fresh ($n = 24$)	0	0	0	24	–	100	–	100
						Norovirus Genogroup II		
Xpert Norovirus ($n = 104$)	41	0	0	63	100	100	100	100
Frozen ($n = 80$)	28	0	0	52	100	100	100	100
Fresh ($n = 24$)	13	0	0	11	100	100	100	100
CerTest Norovirus ($n = 104$)	31	1	10	62	75.6	98.4	96.9	86.1
Frozen ($n = 80$)	21	1	8	50	72.4	98.0	95.5	86.2
Fresh ($n = 24$)	10	0	2	12	83.3	100	100	85.7

[1]Positive predictive value (PPV), Negative predictive value (NPV).
[2]Results obtained in comparison to NVRL reference method. True positives (TP), False positives (FP), False negatives (FN), True negatives (TN).

was identified, and control measures (visual aids, appropriate training/re-training) were implemented to negate the risk of obtaining costly invalid results. There was no observed difference between fresh and frozen sample for the detection of norovirus using Xpert Norovirus. This observation is supported by a recent study analysing the effect of freezing/thawing on norovirus RNA stability in faecal specimens which noticed no reduction in norovirus titre or capsid integrity [20].

The CerTest Norovirus had poorer PPV and NPV values when compared to the Xpert Norovirus assay (Table 1). As reported many times, RT-PCR is superior to RIA for detection of norovirus from faecal specimens [1]. Differences were noted when fresh and frozen faecal specimens tested by CerTest Norovirus were compared. Fresh specimens had higher sensitivity (+13%), specificity (+4.6%), NPV (+6.8%) and PPV (+6.9%) than frozen specimens when results for norovirus for GI and GII were combined. No data from other studies support the observation that freezing faecal specimens decreases CerTest Norovirus performance, especially sensitivity. A recent study tested RIDA®QUICK on 74 freshly collected norovirus, GII positive faecal specimens showed that sensitivity is increased rather than decreased when frozen and thawed. This article only reports for sensitivity when discussing fresh vs. frozen [21]. The sensitivity observed for fresh and thawed specimens was 71 and 78%, respectively [21]. It would be useful for another evaluation of CerTest norovirus to be carried out using a higher sample number to assess if performance characteristics are affected by freeze/thawing of faecal specimens. CerTest Norovirus possesses an excellent combined norovirus PPV value of 94.7% and would be a cost-effective and timely way of large-scale testing, particularly in suspected norovirus outbreak situations. Where CerTest Norovirus testing is negative and clinical suspicion of norovirus infection remains, specimens should be re-tested by the more sensitive Xpert Norovirus assay [19].

The limitations of our study included a relative low number of specimens from patients, limited time frame, limited geographical spread, low number of fresh specimens and low number of norovirus GI positive specimens. Although expensive, Xpert Norovirus is less labour intensive and requires minimal technical expertise compared to high throughput, more technically demanding assays based on molecular genetics [18]. The test is easy to use, rapid (~90 min) and cross-contamination is significantly reduced by the employment of a closed cartridge system [15]. Use of Xpert Norovirus is relevant in the norovirus-testing season where rapid, reliable results are needed to isolate infected patients from non-infected patients [18]. Xpert Norovirus can be utilised in a routine laboratory with low sample numbers, or out of hours in any laboratory setting where a rapid result is required within a short period. To maximise the impact of Xpert Norovirus, MRHT offers a norovirus service to clinicians seven days a week between the hours of 09:00–18:00 (weekdays) and 09:00–14:00 (weekends). The average turnaround time of samples received within this period is less than two hours.

This study is an advance in biomedical science because it shows that the Xpert Norovirus method provides an on-demand, rapid, accurate identification of norovirus genogroups GI and GII in faecal specimens.

Disclosure statement

No potential conflict of interest was reported by the authors.

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