CLINICAL AND VACCINE IMMUNOLOGY, Oct. 2007, p. 1349–1355 Vol. 14, No. 10
1556-6811/07/$08.00+0 doi:10.1128/CVI.00214-07

European Multicenter Evaluation of Commercial Enzyme Immunoassays for Detecting Norovirus Antigen in Fecal Samples▽

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Received 22 May 2007/Returned for modification 16 July 2007/Accepted 15 August 2007

A total of 2,254 fecal samples were tested in a European multicenter evaluation of commercially available norovirus antigen detection assays. Two commercial enzyme immunoassays, IDEIA Norovirus (Oxoid; Thermo Fisher Scientific, Ely, United Kingdom) and RIDASCREEN Norovirus (R-Biopharm, Darmstadt, Germany), were included in the evaluation, and their performance was compared with the results of reverse transcription-PCR (RT-PCR). Included in the evaluation were samples collected in sporadic cases of gastroenteritis, samples from outbreaks in which two or more samples were collected, well-characterized samples representing genotypes currently cocirculating within Europe, and samples collected from patients with gastroenteritis caused by a pathogen other than norovirus. The sensitivities and specificities of the IDEIA Norovirus and RIDASCREEN Norovirus assays were 58.93 and 43.81% and 93.91 and 96.37%, respectively, compared with RT-PCR. The sensitivities of both assays for outbreak investigations improved when six or more samples from an outbreak were examined. The IDEIA Norovirus assay exhibited reactivity to a broader range of norovirus genotypes than the RIDASCREEN Norovirus assay, which showed genotype-dependent sensitivities. The results indicate that, if used, these assays should serve as screening assays and the results should be confirmed by RT-PCR.

Noroviruses (NoVs) are a major cause of nonbacterial gastroenteritis and are associated with outbreaks of diarrheal illness in hospitals (7, 10), nursing and residential homes (9, 13, 15), and other institutional settings. NoV strains exhibit wide genetic diversity, and viruses of both genogroup I (GI) and GII and different genotypes within the genogroups cocirculate in a given geographical region at the same time (8).

Reverse transcription-PCR (RT-PCR) testing is used to identify NoV outbreaks and is now regarded as the "gold standard" (6, 10). However, the genetic diversity of NoVs makes it difficult to design oligonucleotide primers capable of allowing the amplification of sequences from all genotypes in a single assay. Although RT-PCR increases the sensitivity of detection, it may be compromised by the lability of single-stranded viral RNA and difficulties in extracting RNA from fecal samples. Also, the exquisite sensitivity offered by RT-PCR allows the detection of virus in samples from asymptomatic patients and in samples from symptomatic patients whose symptoms are associated with another, coinfecting enteric pathogen.

Antigen detection enzyme immunoassays (EIAs), based on the use of hyperimmune antisera raised against recombinant NoV capsids, are predominantly type specific and may detect only strains of the same or genetically similar genotypes (12). The production of monoclonal antibodies to recombinant NoV capsid proteins (1, 11) allows the construction of a multivalent antibody panel with wide-ranging reactivities capable of detecting a broad range of NoV genotypes within the two NoV genogroups.

Evaluations of three commercially available EIAs [SRSV(II)-AD (Denka Seiken, Chuo-Ku, Japan), IDEIA Norovirus (Dako, Ely, United Kingdom), and RIDASCREEN Norovirus (R-BioPharm, Darmstadt, Germany)] for the detection of NoV antigen have been conducted previously in a number of countries, and several reports have been published (2–5, 17–19). These published evaluations are based on the results of testing 244, 137, 158, 479, 52, 130, and 130 clinical samples, respectively, and give widely varying sensitivities and specificities ranging from <30 to >70% and 69.0 to 100%, respectively. Further unpublished studies undertaken in several European countries have provided equally conflicting results, with calculated sensitivities and specificities ranging from 40 to 92.7% and 40.9 to 97.6%, respectively (personal communication).

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▽ Published ahead of print on 22 August 2007.

TABLE 1. Fecal samples used in the evaluation

Sample group	No. of samples
Samples with NoV strains characterized by sequencing (representative genotypes)	509[a]
Outbreak samples	
2 samples from each of 9 outbreaks	18
3 samples from each of 57 outbreaks	171
4 samples from each of 45 outbreaks	180
5 samples from each of 52 outbreaks	260
6 samples from each of 48 outbreaks	288
7 samples from each of 47 outbreaks	329
>7 samples from each of 15 outbreaks	288
Rotavirus-positive samples	61
Adenovirus-positive samples	22
Astrovirus-positive samples	28
Sapovirus-positive samples	15
C. difficile-positive samples	18
Samples collected in sporadic cases of gastroenteritis	274
Total	2,254

[a] Includes samples from selected outbreaks that are also counted below. The total number of samples tested was 2,191.

These data are difficult to reconcile, as the results of the evaluations may have been affected by several factors, including the diversity of genotypes examined, variable sensitivities of the RT-PCR assays used as the gold standard, sample storage conditions, the numbers of fecal samples examined, and the methods of preanalytical preparation of the samples in each evaluation. Also, newer versions of these kits, for which the manufacturers claim increased sensitivity and specificity, have become available.

A multicenter European evaluation of the IDEIA Norovirus (version 2; Dako, Ely, United Kingdom) and RIDASCREEN Norovirus (R-BioPharm, Darmstadt, Germany) EIAs was undertaken, and samples well characterized as positive or negative for NoV by RT-PCR were analyzed. Discrepant results were resolved through repeat PCR and EIA testing.

MATERIALS AND METHODS

The aims of the study were to determine the statistical agreement between the results obtained with the NoV EIAs and those obtained with the RT-PCR assay, the sensitivities and specificities of the EIAs for determining the etiologies of sporadic cases and outbreaks of gastroenteritis, and the abilities of the EIAs to detect the spectrum of NoV genogroups and genotypes presently cocirculating in the population of Europe. The project was coordinated from the Enteric Virus Unit, Virus Reference Department, Centre for Infections, Health Protection Agency, London, United Kingdom.

Evaluation sites. The multicenter evaluation included laboratories in the United Kingdom, France, Spain, Germany, Italy, and The Netherlands. All participating laboratory personnel had experience performing RT-PCRs for the detection of NoV RNA, and training to familiarize the operators with the EIA methods was provided by the manufacturers. The multicenter evaluation allowed the examination of more samples within a specified time frame and the inclusion of a more diverse range of NoV genotypes than a single-center study.

Samples. A total of 2,254 fecal specimens collected during the 2004-to-2005 and 2005-to-2006 NoV seasons from patients with symptoms of gastroenteritis were included in the evaluation. Samples from a total of 273 outbreaks with various sample group sizes, from 2 to >7 samples per outbreak; 509 samples from which the NoV strains had been characterized by genotyping; 274 samples collected in sporadic cases of gastroenteritis; and 144 samples in which another enteric pathogen had been identified were included in the study (Table 1).

Samples were prepared for testing by each EIA according to the manufacturer's instructions and for testing by RT-PCR and electron microscopy according to validated laboratory protocols.

NoV antigen detection EIAs. The IDEIA Norovirus (Dako) and RIDASCREEN Norovirus (R-BioPharm) EIAs were evaluated against the screening RT-PCR assays currently used in each laboratory (Table 2). EIAs were performed according to the manufacturers' instructions, and RT-PCR assays were performed according to validated laboratory standard operating procedures. All tests were performed with the following manufacturers' lots: IDEIA Norovirus, lot number X025091, and RIDASCREEN, lot number 05245. It should be noted that the IDEIA Norovirus assay used in this evaluation is an update of the previous commercial assay version and that all studies published before the present study report on the earlier version. The latest version of the IDEIA Norovirus assay is a single-plate assay with microwells coated with monoclonal antibodies to both GI and GII NoV strains.

Result analysis. The results obtained from the NoV EIAs were compared with the results of RT-PCR assays by sample and by outbreak. Samples giving discrepant results were retested by both EIAs, and a portion were subjected to a second RT-PCR amplifying a different region of the genome. A proportion of samples still giving discrepant results after retesting were examined by electron microscopy. Positivity of individual samples was assumed if small round structured virus particles, characteristic of NoV, were seen by electron microscopy or if NoV RNA was detected by RT-PCR. Electron microscopy was performed at one site (United Kingdom), and NoV was not detected in any sample that was EIA positive but RT-PCR negative (data not shown; http://www.hpa-standardmethods.org.uk/documents/vsop/pdf/vsop14.pdf). Groups of outbreak samples were deemed positive if NoVs were detected in two or more samples from an outbreak.

Statistical analysis. The sensitivity and specificity of each EIA was determined by comparison with the results of RT-PCR, and 95% confidence intervals

TABLE 2. Oligonucleotide primers for the PCR assays used in the evaluation[a]

Country	Primer(s) used in screening assay	Genome region(s)	Primer(s) used in second assay	Genome region(s)	References
United Kingdom	NiE3	RdRp gene	Orf1/Orf2	RdRp gene-capsid gene region	6, 14
Spain	JV13i/JV12Y	RdRp gene	Orf1/Orf2	RdRp gene-capsid gene region	14, 21
Italy	JV12/JV13	RdRp gene	GI SKF-R/GI SKF-F and GII SKF-R/GII SKF-F	Capsid gene region C	16, 22
France	JV12/JV13	RdRp gene	GI SKF-R/GI SKF-F and GII SKF-R/GII SKF-F	Capsid gene region C	16, 22
The Netherlands	LC1/LC2 real time	RdRp gene-capsid gene region	JV13i/JV12Y	RdRp gene	20, 21

[a] RdRp, RNA-dependent polymerase.

TABLE 3. Sensitivities and specificities of the IDEIA Norovirus and RIDASCREEN Norovirus EIAs compared with RT-PCR

Country	No. of samples	IDEIA Norovirus				RIDASCREEN Norovirus			
		Sensitivity (%)	95% CI	Specificity (%)	95% CI	Sensitivity (%)	95% CI	Specificity (%)	95% CI
France	320	61.36	54.79–67.55	93.81	87.16–97.13	40.37	34.08–46.99	95.96	90.07–98.42
Germany	97	66.67	54.37–77.05	90.32	75.10–96.65	36.51	25.72–48.85	83.33	66.44–92.66
Italy	461	46.81	40.53–53.19	88.05	83.18–91.66	41.70	35.58–48.09	96.90	93.95–98.49
The Netherlands	414	67.43	61.53–72.83	92.67	87.35–95.86	36.33	30.68–42.38	92.00	86.54–95.36
Spain	425	75.86	68.99–81.62	98.01	95.42–99.15	69.54	62.34–75.90	99.20	97.14–99.78
United Kingdom	474	44.63	38.50–50.93	96.73	93.40–98.41	39.50	33.50–45.83	97.61	94.52–98.97
All	2,191	58.93	56.12–61.68	93.91	92.23–95.25	43.81	41.01–46.65	96.37	95.00–97.38

(CI) were calculated (www.healthstrategy.com/epiperl/epiperl.htm). The ability of each EIA to detect a range of genotypes was determined by calculating the percentage of strains of each genotype detected and the 95% CI. Significant differences were confirmed by calculating *P* values using the chi-square test (www.graphpad.com/quickcalcs/contingency1.cfm).

RESULTS

Sensitivities and specificities of the IDEIA Norovirus (Oxoid; Thermo Fisher Scientific) and RIDASCREEN Norovirus (R-Biopharm) EIAs. The results obtained with the positive and negative controls were all within the acceptance criteria stipulated by the manufacturers, and the cutoff values and equivocal ranges were calculated according to the manufacturers' instructions (data not shown).

The sensitivity and specificity of the IDEIA Norovirus assay ranged from 44.63 to 75.86% and 88.05 to 98.01%, respectively, when the results were analyzed by country. Similarly, the sensitivity and specificity of the RIDASCREEN assay ranged from 36.33 to 69.54% and 83.33 to 99.20%, respectively (Table 3). The analysis of the total data set gave sensitivities for the IDEIA Norovirus and RIDASCREEN assays of 58.93 and 43.81%, respectively, and specificities of 93.91 and 96.37%, respectively (Table 3). Equivocal results were excluded from the analysis of sensitivity and specificity, but when they were included as representing either a positive or a negative result, no significant change was detected (data not shown).

The median number of samples examined per outbreak was 5 (range, 2 to 39). Compared with RT-PCR, the sensitivities of the IDEIA Norovirus and RIDASCREEN assays for determining the cause of an outbreak by detecting NoV in two or more samples were 65.90% (95% CI, 59.36 to 71.88%) and 55.30% (95% CI, 48.65 to 61.77%), respectively, when results from two or more samples per outbreak were analyzed (Table 4). The specificities of the IDEIA Norovirus and RIDASCREEN assays were 95.65% (95% CI, 87.98 to 98.51%) and 97.10% (95% CI, 90.03 to 99.20%), respectively (Table 4). The sensitivities and specificities for detecting NoV antigen in specimens collected in sporadic cases of gastroenteritis were 46.21% (95% CI, 36.63 to 56.29%) and 95.72% (95% CI, 91.79 to 97.82%) for the IDEIA Norovirus assay and 31.58% (95% CI, 23.10 to 41.49%) and 99.47% (95% CI, 97.03 to 99.91%) for the RIDASCREEN assay.

The abilities of the IDEIA Norovirus and RIDASCREEN assays to detect NoV in two or more samples per outbreak were determined for all outbreaks and for outbreaks from which three to more than seven samples were received (Table 4). The IDEIA Norovirus assay demonstrated a statistically significant increase in the number of NoV outbreaks detected when six samples per outbreak were tested rather than only

TABLE 4. Abilities of the IDEIA Norovirus and RIDASCREEN Norovirus EIAs to detect NoVs in RT-PCR-positive samples collected in sporadic cases and in two or more positive samples from outbreaks

Sample sources	IDEIA Norovirus				RIDASCREEN Norovirus			
	Sensitivity (%)	95% CI	Specificity (%)	95% CI	Sensitivity (%)	95% CI	Specificity (%)	95% CI
Sporadic infections	46.21	36.63–56.29	95.72	91.79–97.82	31.58	23.10–41.49	99.47	97.03–99.91
All outbreaks	65.90	59.36–71.88	95.65	87.98–98.51	55.30	48.65–61.77	97.10	90.03–99.20
Outbreaks in which the no. of samples per outbreak collected was:								
2	33.33	12.06–64.58	100.00	56.55–100.0	44.44	18.88–73.33	100.00	56.55–100.0
3	46.34	32.06–61.25	94.12	73.02–98.95	29.27	17.61–44.48	88.24	65.66–96.71
4	72.73	55.78–84.93	85.71	60.06–95.99	60.61	43.68–75.32	100.00	78.47–100.0
5	55.26	39.71–69.85	100.00	80.64–100.0	55.26	39.71–69.85	100.00	80.64–100.0
6	76.92	61.66–87.35	100.00	74.12–100.0	58.97	43.42–72.92	100.00	74.12–100.0
7	80.95	66.70–90.02	100.00	60.97–100.0	66.67	51.55–78.99	100.00	60.97–100.0
>7	80.00	54.81–92.95			80.00	54.81–92.95		

TABLE 5. PPVs and NPVs[a]

Sample group	Test	No. of samples					PPV (%)	NPV (%)
		Total	Positive by PCR and EIA	Positive by EIA only	Negative by PCR and EIA	Negative by EIA only		
All samples	IDEIA	2,167	706	59	910	492	92.30	64.90
	RIDASCREEN	2,152	520	35	930	667	93.70	58.20
Samples collected in sporadic cases	IDEIA	274	41	8	179	46	83.60	79.50
	RIDASCREEN	274	27	1	186	60	96.42	75.60
Samples from all outbreaks	IDEIA	273	135	3	65	70	97.82	48.14
	RIDASCREEN	273	115	2	66	90	98.29	42.30
Samples from outbreaks with:								
<6 samples per outbreak	IDEIA	173	67	3	49	54	96.60	43.90
	RIDASCREEN	173	57	2	50	64	95.70	47.60
≥6 samples per outbreak	IDEIA	113	76	0	17	20	100.00	45.90
	RIDASCREEN	113	63	0	17	33	100.00	34.00

[a] Total number of samples tested, 2,191; number with equivocal IDEIA results, 24; number with equivocal RIDASCREEN results, 42.

three ($z = \pm 3.191$; $P = 0.0014$). Similarly, the RIDASCREEN assay identified a significantly larger number of outbreaks as being caused by NoV when seven samples per outbreak were tested than when only three samples were available for testing ($z = \pm 3.828$; $P = 0.0001$) (Table 4).

The positive predictive values (PPVs) and the negative predictive values (NPVs) of the IDEIA Norovirus assay were 92.3 and 64.9%, respectively, when the results for individual samples were analyzed and 97.9 and 47.10%, respectively, when the results for outbreaks were analyzed (Table 5). Similarly, the PPVs and NPVs of the RIDASCREEN assay were 93.7 and 58.2%, respectively, for individual samples and 98.4 and 40.9%, respectively, for outbreaks.

Detection of diverse genotypes. The NoV genogroups and genotypes of strains in 509 samples were determined, and the proportions of different genotypes broadly represented those observed to cocirculate in the population. GII genotype 4 (GII-4) strains were the most common strains included, but significant numbers of GI-2, GI-3, GII-3, GII-7, and recombinant GII (rGII) strains, resulting predominantly from recombination between GII-1 and GII-3, were also included. The IDEIA Norovirus assay was able to detect a wide range of genotypes within GI and GII, whereas the RIDASCREEN assay detected a much narrower range of genotypes within GI and GII (Table 6).

Nonspecific reactivity. Nonspecific reactivity was detected in two samples tested with the IDEIA Norovirus assay. *Clostridium difficile* had been detected previously in one sample, and enteric adenovirus had been detected in the other. Repeat RT-PCR testing could not confirm the presence of NoV RNA in these samples (Table 7), although both samples were reactive when subjected to repeated testing by the EIA.

TABLE 6. Detection of strains representative of NoV genotypes within GI, GII, and GIV

NoV classification	No. of positive samples	IDEIA Norovirus		RIDASCREEN Norovirus		*P* value[a]
		No. (%) of samples in which genotype was detected	95% CI	No. (%) of samples in which genotype was detected	95% CI	
GI-1	5	4 (80.00)	37.55–96.36	3 (60.00)	23.07–88.24	0.49
GI-2	13	11 (84.62)	57.77–95.67	2 (15.38)	4.33–42.23	**0.0002**
GI-3	28	12 (42.86)	26.51–60.93	9 (32.14)	17.93–50.66	0.4
GI-4	2	2 (100.00)	34.24–100.0	0 (0.00)	0.00–65.76	0.3
GI-5	8	3 (37.50)	13.68–69.43	0 (0.00)	0.00–32.44	0.2
GI-6	7	5 (71.43)	35.89–91.78	0 (0.00)	0.00–35.43	**0.02**
GI-7	1	0 (0.00)	0.00–79.35	0 (0.00)	0.00–79.35	>0.5
GII-1	8	7 (87.50)	52.91–97.76	0 (0.00)	0.00–32.44	**0.0024**
GII-2	16	8 (50.00)	28.00–72.00	4 (25.00)	10.18–49.50	0.2
GII-3	52	30 (57.69)	44.19–70.13	11 (21.15)	12.24–34.03	**0.0003**
GII-4	301	203 (67.44)	61.96–72.49	186 (61.79)	56.19–67.10	0.17
GII-5	6	2 (33.33)	9.68–70.00	1 (16.67)	3.01–56.35	>0.5
GII-6	9	2 (22.22)	6.32–54.74	0 (0.00)	0.00–29.91	0.4
GII-7	29	20 (68.97)	50.77–82.72	5 (17.24)	7.60–34.55	**0.002**
GII-8	1	0 (0.00)	0.00–79.35	0 (0.00)	0.00–79.35	>0.5
GIV-1	4	0 (0.00)	0.00–48.99	0 (0.00)	0.00–48.99	>0.5
rGII	19	10 (52.63)	31.71–72.67	2 (10.53)	2.94–31.39	**0.01**

[a] *P* values indicating significance are in boldface.

TABLE 7. Nonspecific reactivity detected with the EIAs and results of RT-PCR tests to confirm the presence of NoV

Organism	No. of samples			
	Positive for organism	Positive by IDEIA EIA	Positive by RIDASCREEN EIA	Confirmed positive by PCR
Astrovirus	28	1	0	1
C. difficile	18	1	0	0
Enteric adenovirus	22	2	0	1
Rotavirus	61	2	1	2
Sapovirus	15	3	0	3

DISCUSSION

The comparability of assays for the detection of NoV in fecal samples is difficult to determine. Electron microscopy, which is subjective and relatively insensitive, relies on the identification of particles with the characteristic NoV morphology. Antigen detection relies on the presence of, and reactivity with, a cocktail of serotype-specific or cross-reactive antibodies, and genome detection requires the extraction of labile single-stranded RNA and the use of broadly reactive oligonucleotide primers. Also, the heterogeneous and complex nature of a fecal sample may result in differences in the ability to detect the virus. Storage conditions may have altered the virus morphology; blood, often associated with nonspecific reactivity in immunological assays, may be present in the feces; or inhibitors of PCR may not be removed during processing. Therefore, any evaluation of one methodology using another as a gold standard must be powered sufficiently to mitigate the different characteristics of the detection methods and the variability associated with a heterogeneous sample.

A study in which 50% of the fecal samples were positive and which was performed at more than one site (clustered study) would reach the 95% confidence level when >780 samples were tested. In this study, a sample size of >2,000 fecal samples was chosen to take into account not only the performance of testing at multiple sites but also the potential for multiple genotypes of the virus to be detected with different degrees of efficiency. This sample size and multicenter approach also allowed for comparisons among countries in which different mixes of virus genotypes may have been circulating and comparisons with the results of previous studies carried out in those countries.

Overall, the sensitivity of the IDEIA Norovirus assay was significantly higher than that of the RIDASCREEN Norovirus assay, and although a higher specificity was measured with the RIDASCREEN Norovirus assay in some countries, this difference was not statistically significant. The sensitivities of the IDEIA and RIDASCREEN Norovirus assays ranged from 44.63 to 75.86% and 36.33 to 69.54%, respectively, when the results were analyzed by country. The variation in the IDEIA Norovirus assay was characterized by the measurement of significantly lower sensitivities in Italy and the United Kingdom than in all the other countries and a significantly higher sensitivity in Spain than in France, Italy, and the United Kingdom. Similarly, the sensitivity of the RIDASCREEN Norovirus assay was significantly higher in Spain than in all other countries.

A comparison of data from previously published studies, unpublished data, and data from this study, analyzed using the same method, which takes into account the numbers of false-negative results (sensitivity) and false-positive results (specificity) and incorporates the 95% CI, is shown in Table 8. Significant differences in sensitivity were detected when the results of the published studies of the IDEIA Norovirus version 1 assay were analyzed (Table 8). Results of previous studies of the IDEIA Norovirus version 1 assay by Richards et al. and Dimitriadis et al. (4, 5, 18) were comparable and showed higher sensitivity than the results of studies by Rabenau et al., Burton-MacLeod et al., and de Bruin et al (2, 3, 17). The IDEIA Norovirus version 2 assay was significantly more sensitive than the version 1 assay in The Netherlands but was significantly less sensitive when tested in the United Kingdom. There was no statistically significant difference in the specificities of the two IDEIA Norovirus assays.

The results of recent studies performed with the RIDASCREEN Norovirus assay in the United Kingdom and The Netherlands were comparable, with no significant differences in sensitivity or specificity. Interestingly, the results of one study performed in Australia (4) and previously unpublished data from the United Kingdom showed significantly higher sensitivities than the results of recent studies performed in the United Kingdom (18), Germany (19), and The Netherlands (3) (Table 8).

The failure to detect NoVs in clinical samples is often associated with the wide genetic and antigenic diversity of NoV strains. The RIDASCREEN assay was statistically significantly less able to detect GI-2, GI-6, GII-1, GII-3, GII-7, and rGII strains than the IDEIA Norovirus assay (Table 6). The RIDASCREEN assay was unable to detect GI-4, GI-5, and GII-6 strains, and both assays failed to detect GI-7, GII-8, and GIV-1 strains, although the number of samples containing these genotypes was too small to reach statistical significance (Table 6). Also, among the genotypes detected, the IDEIA Norovirus assay was able to detect a higher proportion of the strains within a genotype than the RIDASCREEN assay (Table 6). Interestingly, this difference was less pronounced for GII-4 strains, which were detected by the IDEIA Norovirus assay and the RIDASCREEN Norovirus assay in 67.44 and 61.79% of the RT-PCR-positive GII-4 samples, respectively (Table 6). This finding would suggest that the RIDASCREEN assay has a higher sensitivity for GII-4 strains than for those of other genotypes. The analysis of the results for 299 GII-4 strains and 206 non-GII-4 strains indicated that the sensitivities of detection of GII-4 strains and non-GII-4 strains by the RIDASCREEN assay were significantly different (GII-4, 61.87% [95% CI, 56.25 to 67.19%]; non-GII-4, 34.47% [95% CI, 28.31 to 41.19%]) from the sensitivities of detection of these strains by the IDEIA Norovirus assay (GII-4, 67.57% [95% CI, 62.04 to 72.65%]; non-GII-4, 65.22% [95% CI, 58.51 to 71.37%]). This suggests that differences in sensitivity values obtained in different countries are likely to be associated with the mixes of genotypes included in the evaluation panels. It was not possible to have a set of standard methods for nucleic acid extraction, RT, and PCR used in all countries or source reagents from a single supplier, and for this reason it was decided that a second PCR amplifying another region of the NoV genome would be included in the protocol in order to mitigate the differences in sensitivity measurements associated with a

TABLE 8. Comparison of the results of this study with those from previously published and unpublished studies[a]

Study (reference)	No. of samples tested by IDEIA Norovirus version 1	IDEIA Norovirus version 1				No. of samples tested by IDEIA Norovirus version 2	IDEIA Norovirus version 2			No. of samples tested by RIDASCREEN Norovirus	RIDASCREEN Norovirus			
		Sens (%)	95% CI	Spec (%)	95% CI		Sens (%)	95% CI	Spec (%)	95% CI		Sens (%)	95% CI	Spec (%)	95% CI
Richards et al., 2003, UK (18)	479	55.50	51.10–60.00	98.30	97.1–99.9										
Unpublished study, 2004, UK[b]											346	64.00	52.70–73.94	42.44	36.70–48.39
Schmid et al., 2004 (19)											52	34.62	19.41–53.78	65.38	46.22–80.59
Rabenau et al., 2003 (17)	244	31.25	19.95–45.33	94.90	90.86–97.21										
Burton-MacLeod et al., 2004 (2)	130	39.80	30.67–49.70	100.0	89.28–100.0										
Dimitriadis and Marshall, 2005 (4)											137	71.00	61.46–78.99	46.67	30.23–63.86
Dimitriadis et al., 2006 (5)	130	62.89	52.95–71.84	87.88	72.67–95.18										
de Bruin et al., 2006, NL (3)	158	37.84	27.65–49.23	96.43	90.02–98.78						158	36.49	26.44–47.87	88.10	79.46–93.40
This study, 2006, NL samples						414	67.43	61.53–72.83	92.67	87.35–95.86	414	36.33	30.68–42.38	92.00	86.54–95.36
This study, 2006, UK samples						474	44.63	38.50–50.93	96.73	93.40–98.41	474	39.50	33.50–45.83	97.61	94.52–98.97

[a] Sens, sensitivity; Spec, specificity; NL, The Netherlands; UK, United Kingdom.
[b] Sensitivity and specificity were determined from a total of 346 fecal samples, 204 NoV RT-PCR-positive and 142 RT-PCR-negative samples.

single PCR. Also, the incorporation of a second PCR would mitigate differences in the efficiency of amplification of different genogroups and even genotypes. PCRs targeting the *Orf1-Orf2* junction region and used in each of the countries were likely to amplify genogroups with the same efficiency, as genogroup-specific assays were used, and also genotypes as there is a high degree of conservation within this region among the genogroups.

The sensitivities of the IDEIA Norovirus and the RIDASCREEN assays for detecting NoV in samples collected in sporadic cases of gastroenteritis were 46.32 and 31.58%, respectively. The improvement in the ability of each of the assays to identify the cause of an outbreak when increased numbers of samples per outbreak were tested and the sensitivity and specificity results for samples collected in sporadic cases suggest that these assays are inappropriate for the detection of NoV in samples collected in sporadic cases of gastroenteritis.

It is clear from the results of this study and other published studies that both the IDEIA Norovirus and the RIDASCREEN assays have limitations when used to detect NoV in clinical samples. They should be regarded as screening assays which have the benefit of speed and, in many instances, can be performed closer to the patient or the site of the outbreak. The confidence provided by the detection of NoV antigen in multiple samples collected during an outbreak suggests that when a sufficient number of samples (six or more) were collected, these assays would be appropriate for use in the military, on cruise ships, and during institutional outbreaks in hospitals or nursing homes. Nevertheless, they should be used in conjunction with RT-PCR and proper referral patterns established in order to confirm positive findings and further test negative samples.

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