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ScienceDirect
Journal of Virological Methods 137 (2006) 259–264
Journal of Virological Methods
www.elsevier.com/locate/jviromet

Diagnosis of Norovirus outbreaks by commercial ELISA or RT-PCR

Erwin de Bruin *, Erwin Duizer, Harry Vennema, Marion P.G. Koopmans

Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, National Institute for Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands

Received 2 March 2006; received in revised form 14 June 2006; accepted 22 June 2006
Available online 9 August 2006

Abstract

The IDEIA Norwalk-like virus (Dakocytomation Ltd., Ely, United Kingdom) and the Ridascreen Norwalk-like virus enzyme immunoassay (R-Biopharm AG, Darmstadt, Germany), were evaluated for the diagnosis of outbreaks of acute gastroenteritis.

A panel of 158 fecal samples from 23 outbreaks, including confirmed rotavirus and astrovirus outbreaks, was used to determine the sensitivity and specificity of both ELISA kits relative to an RT-PCR protocol that was followed by Southern blot hybridization. Another panel consisted of 6 different genogroup I strains, 12 genogroup II strains and 1 genogroup IV strain and was used to determine the scope of the tests.

Compared to the RT-PCR, sensitivities of 38% and 36% and specificities of 96% and 88% were found for the Dako kit and the Ridascreen kit, respectively. Two genogroup I strains, and one genogroup II strain were not detected by the Dako kit, while five genogroup I and five genogroup II strains were not detected by the Ridascreen kit.

The sensitivity of both ELISA kits, and the scope of the Ridascreen are considered disappointing. However, the ELISA kits can be useful for a preliminary screening, provided that ELISA negative outbreaks will be re-tested by RT-PCR methods.

Keywords: ELISA; Caliciviridae; Non-bacterial gastroenteritis; Norovirus

1. Introduction

Viral agents are the leading cause of gastroenteritis, with Norovirus (NoV) as the most common pathogen in almost all age groups (de Wit et al., 2001). NoV is associated commonly with large outbreaks in hospitals, homes for the elderly and other institutional settings, especially in the winter months. Transmission of NoV occurs by the fecal–oral route and is transmitted mostly person to person but may also be associated with contaminated food or water (Green et al., 2001).

NoVs are a group of genetically diverse RNA viruses in the family Caliciviridae. Currently, based on the diversity of the capsid sequences, NoVs are grouped into five genogroups, of which three have been found in humans; genogroups I, II and IV (Koopmans et al., 2003).

Characterization of NoV has been hindered by the lack of a cell culture system or a small animal model (Duizer et al., 2004; Rockx et al., 2005). For many years NoV has been diagnosed using electron microscopy (EM), reverse transcription polymerase chain reaction (RT-PCR), and "in-house" enzyme-linked immunosorbent assays (ELISA) (Atmar and Estes, 2001; Rabenau et al., 2003). EM is a relatively insensitive method with a detection limit of approximately 10^6 NoV particles per gram of feces and it requires skilled personnel and sophisticated equipment (Atmar and Estes, 2001). ELISAs are generally easy to perform without the need for sophisticated equipment. However, due to the lack of sufficient quantities and quality of NoV antigens the availability of ELISAs for NoV detection has been restricted to in-house assays rather than assays that are available commercially (Vipond et al., 2000). Currently, the detection method used most widely is RT-PCR, however, several investigations are developing modification of this molecular detection method and methods such as real-time-RT-PCR, or multiplex-real-time RT-PCR protocols have been published (Richards et al., 2004; Mohamed et al., 2006). These methods can be highly sensitive and specific, but detection of viral agents with molecular techniques also requires operator skills and sophisticated equipment. The use of highly purified antigens produced by molecular cloning techniques has resulted in the availability of

* Corresponding author. Tel.: +31 30 274 3738; fax: +31 30 274 4418.
E-mail addresses: Erwin.de.bruin@rivm.nl (E. de Bruin), erwin.duizer@rivm.nl (E. Duizer), harry.vennema@rivm.nl (H. Vennema), marion.koopmans@rivm.nl (M.P.G. Koopmans).

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doi:10.1016/j.jviromet.2006.06.024

commercial kits, and consequently more frequent use of ELISA techniques for the detection of NoV (Burton-MacLeod et al., 2004; Dimitriadis and Marshall, 2005; Richards et al., 2003; Rabenau et al., 2003).

To the best of our knowledge this is the first report evaluating these two commercial ELISA tests with identical sample sets. The IDEIA NLV kit (DakoCytomation Ltd., Ely, UK) and the Ridascreen Norwalk-like virus EIA (R-biopharm AG, Darmstadt, Germany) and an RT-PCR method which is used extensively (Vennema et al., 2002) were used to test fresh and stored stool samples for the presence of NoV. The sensitivity and specificity of both ELISA kits were determined, using a common RT-PCR assay as the "gold standard".

2. Materials and methods

2.1. Fecal samples

In this study, two panels of clinical samples were used. The first panel of 158 fecal samples from 23 different outbreaks was used to measure the sensitivity and specificity of both ELISAs for diagnosis of a gastroenteritis outbreak (Table 1). The samples were from outbreaks of gastroenteritis that had been tested for NoV by RT-PCR in 2002 and 2003; this panel included specimens from one outbreak that were found positive for Rotavirus and Astrovirus, two outbreaks that were found positive for Rotavirus only, and one that was positive for Astrovirus only. Stool samples were stored as 10–20% suspensions in Medium 199 with Hanks' salts and 25 mg/l gentamycine. All stool samples were tested directly or stored at 4 °C with a maximum of 2 months for the Dako kit. The samples were stored at 4 °C and tested within 2 years by the Ridascreen kit.

The second panel was composed to represent a wide variety of NoV lineages to evaluate the scope of the diagnostic tests. It consisted of 19 samples that were found positive for NoV by RT-PCR. The NoV lineages were typed by partial sequencing of ORF 2. These stool specimens were collected from the Dutch gastroenteritis surveillance from 1999 to 2002. This panel contained 6 samples representing five genogroup I genotypes, 12 samples representing six genogroup II genotypes, and one genogroup IV strain (Table 2). Stool samples were stored as 10–20% suspensions in Medium 199 with Hanks' salts and 25 mg/l gentamycine at −80 °C until use.

2.2. RNA extraction

RNA was extracted from 100 μl of a 10–20% stool suspension by binding to size-fractionated silica particles in the presence of guanidinium isothiocyanate (GuSCN) and Triton-X100 (Boom et al., 1990). After centrifugation (1 min. at 13,000 × *g*), the pellet was washed twice with a 500 μl GuSCN containing washing buffer, twice with 500 μl ethanol 70% (v/v), and once with 500 μl acetone. After removal of acetone by evaporation, the RNA was eluted in 25 μl distilled, DNAse RNase free water with 0.1 mM DTT and RNAguard (250 units/ml) (Promega, Lei-

Table 1
Detection of human NoV in stool samples with in-house RT-PCR and two commercial ELISA kits

Year	Outbreak number	Sequence type[a]	No. of samples	No. of samples tested positive (%)				
				PCR	Dako GGI	Dako GGII	Dako both	Ridascreen
2002	173	GGII.4	16	10 (63)	0 (0)	1 (6)	1 (6)	10 (63)
	203	GGII.4	8	6 (75)	0 (0)	0 (0)	0 (0)	0 (0)
	206	GGII.4	15	5 (33)	0 (0)	3 (20)	3 (20)	2 (13)
	215	GGII.4	7	7 (100)	0 (0)	7 (100)	7 (100)	3 (43)
	222	GGII.4	14	8 (57)	0 (0)	2 (14)	2 (14)	2 (14)
	227	GGII.4	6	5 (83)	0 (0)	0 (0)	0 (0)	1 (16)
	229	GGII.4	4	3 (75)	1 (25)	2 (50)	3 (75)	1 (25)
2003	13	Rotavirus	5	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)
	28	Astrovirus	5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	37	Rota- and Astrovirus	7	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	48	Rotavirus	4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	53	GGII.4	6	6 (100)	0 (0)	4 (67)	4 (67)	6 (100)
	54	GGII.7	9	4 (44)	0 (0)	2 (22)	2 (22)	2 (22)
	55	GGI.6	4	2 (50)	0 (0)	0 (0)	0 (0)	2 (50)
	56	GGI.6	5	3 (60)	3 (60)	0 (0)	3 (60)	3 (60)
	57[b]	GGII.b	7	1 (14)	0 (0)	1 (14)	1 (14)	0 (0)
	58	GGII.4	5	2 (40)	0 (0)	1 (20)	1 (20)	2 (40)
	59		2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	60		6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	62		5	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)
	70	GGI.6	4	3 (75)	3 (75)	1 (25)	3 (75)	0 (0)
	71	GGII.2	3	3 (100)	0 (0)	0 (0)	0 (0)	1 (33)
	73	GGII.b	11	6 (55)	0 (0)	1 (9)	1 (9)	0 (0)
Total			158	74 (47)	7 (4)	25 (16)	31 (20)	37 (23)

[a] Based on RNA dependent RNA polymerase sequence data.
[b] Outbreak number 57 of the year 2003 is not assigned as a NoV positive outbreak.

Table 2
Test results of the panel of NoV positive samples confirmed by RT-PCR, used to evaluate the breadth of the ELISA kits

Strain	Genotype[a]	PCR	Dako GGI	Dako GGII	Ridascreen
Hu/NV/IJsselstein57/2001/NL	GGI.2	+	+	−	+
Hu/NV/Driebergen45/2001/NL	GGI.3	+	+	−	−
Hu/NV/NieuwLekkerland36/2000/NL	GGI.3	+	−	−	−
Hu/NV/Koudekerke38/2000/NL	GGI.4	+	+	−	−
Hu/NV/Veendam05/2002/NL	GGI.5	+	−	−	−
Hu/NV/DenHaag45/2000/NL	GGI.7	+	+	−	−
Hu/NV/DenHaag08/2001/NL	GGII.1	+	+	+	−
Hu/NV/DenHaag09/2001/NL	GGII.1	+	+	−	−
Hu/NV/Rotterdam29/2001/NL	GGII.1	+	−	+	+
Hu/NV/Delft48M/2000/NL	GGII.2	+	+	−	+
Hu/NV/Haarlem54/2001/NL	GGII.2	+	+	−	+
Hu/NV/Delft48R/2000/NL	GGII.3	+	−	+	−
Hu/NV/Amsterdam14/1999/NL	GGII.3	+	+	+	−
Hu/NV/Coslada16/1999/SP	GGII.3	+	−	+	−
Hu/NV/Houten35/2001/NL	GGII.4	+	−	+	+
Hu/NV/Zeeland26/2000/NL	GGII.4	+	+	+	+
Hu/NV/Explosie13/2001/NL	GGII.4	+	−	−	+
Hu/NV/DenHaag47/2000/NL	GGII.5	+	−	+	+
Hu/NV/Veghel12/2000/NL	GGIV[b]	+	+	−	−

[a] The genetic clusters shown are based on criteria described by Green et al. (2001). Where the roman character represents the genogroup and the second number represents the genetic cluster.
[b] Genogroup IV is a prospective genogroup for the Alphatron/1998/NL-cluster.

den, The Netherlands). The extracted RNA was either used directly in RT-PCR or stored at −70 °C.

2.3. RT-PCR protocol

The RT-PCR was carried out as described previously (Vennema et al., 2002). Clinical samples were screened routinely with the primers targeting the RNA polymerase region. The RT-PCR for the capsid region is not tested routinely. Reverse transcription of 2.5 μl of the extracted RNA was done for 60 min at 42 °C after annealing with RT-primer (Table 3). Reaction was performed in a 15 μl reaction containing 0.3 μM primer JV13I, 10 mM Tris–HCl pH 8.3, 50 mM KCl, 3 mM $MgCl_2$, 1 mM dNTP and 5 U AMV-RT (Promega, Leiden, The Netherlands). Five microliters of the RT-mix was added to 45 μl of a PCR-mix containing 10 mM Tris–HCl pH 9.2, 50 mM KCl, 1.2 mM $MgCl_2$ (final concentration 1.5 mM), 0.2 mM dNTPs, 2.5 units ampliTaq and 0.3 μM of PCR-primer (Table 3). Samples were denatured for 3 min at 94 °C and subjected to 40 cycles at 94 °C for 1 min, 37 °C for 1 min 30 s, and 72 °C for 1 min. The low annealing temperature is vital to allow binding to a wide range of viral sequences. The amplification products were analyzed by 2% agarose gel electrophoresis and visualized with UV after ethidium bromide staining.

2.4. Southern blot hybridisation

Confirmation of RT-PCR positive result was done by Southern blot hybridization. The RT-PCR products in the agarose gel were denatured by incubating in 0.5 M NaOH for 30 min and transferred to a nylon membrane with positive charge by vacuum blotting. Hybridization of NoV RT-PCR products was performed as described previously (Vinje et al., 1997). Briefly, the nylon membranes were prehybridised for 30 min at 42 °C in

Table 3
Primers used for detection of NoV in stool specimen

Function of primer	Primer name	Sequence (5′ to 3′)	Target
RT-primers	JV13I	TCA TCA TCA CCA TAG AAI GAG	ORF 1
	JV21	CCN RCM YAA CCA TTR TAC AT	ORF 2 (GGII)
	JV23	ATA TTI CCM ACC CAR CCA TT	ORF 2 (GGI)
PCR-primers	JV12Y	ATA CCA CTA TGA TGC AGA YTA	ORF 1
	JV22	GTA AAT GAT GAT GGC GTC TA	ORF 2 (GGI)
	JV24	GTG AAT GAA GAT GGC GTC GA	ORF 2 (GGII)
Sequence primer	JV12S	ATA CCA CTA TGA TGC AGA	ORF 1
Probes	UK3	GTC CCC TGA CAT CAT ACA GGC T	ORF 1
	JV5	CTC ACC AGA GGT TGT CCA AGC	ORF 1
	GGI	ATG GAY GTT GGS GAY TAY RT	ORF 1
	GGII	GAA YTC CAT CRC CCA YTG	ORF 1

20 ml 2× SSPE (300 mM NaCl, 20 mM $NaH_2PO_4H_2O$, 2 mM EDTA, pH 7.4) with 0.1% SDS. Twenty pmol of each of the 5′-biotinylated probes (Table 3) were added and the membranes were left for 45 min at 42 °C to allow hybridization. The membranes were washed three times for 10 min at 42 °C with 2× SSPE and 0.1% SDS. Subsequently, the membrane was incubated with 1:4000 diluted streptavidin-peroxidase conjugate for 45 min at 42 °C in 10 ml of 2× SSPE and 0.5% SDS. After washing three times (10 min each) with decreasing concentration of SDS (0.5%, 0.1%, and 0%) in 2× SSPE, the membranes were incubated for 1 min with the enhanced chemoluminescence (ECL) detection reagent, and then exposed to an ECL hyperfilm for 15 min.

2.5. Sequencing

Exact typing of the RT-PCR positive and Southern blot hybridization confirmed samples was carried out by sequencing. Sequencing of the RT-PCR products was carried out with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, USA) on an automated sequencer (Applied Biosystems model 3700) using the PCR primer (Table 3). DNA sequences were edited using SeqMan (DNAStar Inc., Konstanz, Germany). Genetic classification of the strains was performed using the program Bionumerics (Applied Maths BVBA, Sint-Martens-Latem, Belgium).

2.6. NoV ELISAs

The Dako kit for detection of NoV in stool specimen (IDEIA™ Norwalk-like virus (NLV), Dakocytomation Ltd., Ely, United Kingdom; K6043 two-plate version) utilizes microwells coated with genogroup I or genogroup II specific monoclonal antibodies. The assay was carried out according to the manufacturer's instructions. Briefly, 100 μl 10% fecal suspension or control sample (provided by the kit) was added to the microwell and incubated simultaneously with 100 μl specific genogroup I or genogroup II polyclonal antibody conjugated to horseradish peroxidase. After 120 min incubation the microwells were washed five times with working strength washing buffer. The presence of specifically bound enzyme labeled antibody was determined by addition of 100 μl aqueous solution of 3,3′-, 5,5′-tetramethylbenzidine and hydrogen peroxidase. After 30 min incubation at room temperature the $A_{450\,nm}$ was measured using $A_{620\,nm}$ as reference. Any clinical specimen with an $A_{450\,nm}$ greater than the calculated cut-off value is positive. The cut-off value was calculated by adding 0.10 absorbance units to the negative control value, according to manufacturers instructions.

The Ridascreen kit for detection of NoV in stool specimen (Norwalk-like virus enzyme immunoassay, R-Biopharm AG, Darmstadt, Germany; article number C1401 with production date March 2004) utilizes microwell strips coated with specific antibodies against antigens of several different genotypes. This assay was also carried out according to manufacturer's instructions. Briefly, samples and controls were pipetted into the wells and then incubated at room temperature for 1 h. After washing the wells five times with 300 μl working strength washing buffer, specific antibodies against NoV conjugated to horseradish peroxidase were added. After 30 min incubation the microwells were washed five times with 300 μl working strength washing buffer. Substrate/chromogen was added to the microwells and incubated 15 min at room temperature. After 15 min the reaction was stopped by adding Stop solution and the $A_{450\,nm}$ was measured using $A_{620\,nm}$ as reference. The cut-off value was calculated by adding 0.15 absorbance to the mean value of the negative control (according to manufacturer's instructions). Samples which have an absorbance value within 10% above or below the threshold were re-tested.

3. Results

3.1. Agreement between ELISAs and RT-PCR

A total of 158 samples from 23 different outbreaks (panel 1) were collected and analyzed using antigen ELISA kits and RT-PCR. A median of 6, ranging from 2 to 16, samples were tested per outbreak (Table 1). Ten (6%) stool specimen were positive in RT-PCR and both ELISA kits, 71 (45%) were negative by all tests and 77 (49%) samples gave discrepant results. No samples were positive by both ELISA kits and negative by RT-PCR. Three samples from 3 outbreaks were only positive by the Dako kit, 10 samples from 7 outbreaks were only positive by the Ridascreen kit and 29 samples from 10 outbreaks were positive only by RT-PCR. This gave an overall agreement of 51% between all three tests.

3.2. Sensitivity and specificity

In panel 1, 74/158 (47%) samples were positive by RT-PCR, confirmed by Southern blot hybridization and sequencing. When RT-PCR is regarded as the "gold standard", the Dako kit detected 28/74 (38%) positive samples and the Ridascreen kit detected 27/74 (36%) positive samples. A total of 84/158 (53%) samples were tested negative by RT-PCR and confirmed by Southern blotting. From these 84 samples, the Dako kit detected 3 (4%) NoV positive samples and the Ridascreen kit detected 10 (12%) NoV positive samples, resulting in specificities of 96% and 88% for the Dako kit and the Ridascreen kit, respectively.

3.3. Scope of tests

The second panel of 19 RT-PCR NoV positive samples from 6 different genogroup I strains, 12 genogroup II strains and a genogroup IV, were all tested by both ELISA kits. Four of 6 (67%) strains of genogroup I, 11 of 12 (92%) representative strains of NoV genogroup II and the genogroup IV strain were detected with the Dako kit. The genogroup I test from Dako kit cross-reacted with 6 out of 12 (50%) genogroup II strains (subtypes; GGII 1, 2, 3 and 4). The genogroup II test did not cross-react with genogroup I stool specimens. The genogroup IV sample did react with the genogroup I test, but not with the genogroup II test (Table 2).

Table 4
Number of NoV positive samples from outbreaks tested NoV positive by RT-PCR

Diagnostic test	No. of positive samples in NoV outbreaks (%)		No. of positive outbreaks (%)	
	Criterium A^I ($n=73$)	Criterium B^I ($n=62$)	Criterium A^I ($n=15$)	Criterium B^I ($n=12$)
Dako kit	30 (26)	24 (27)	8 (53)	5 (42)
Ridascreen kit	35 (30)	29 (33)	9 (60)	4 (33)

A^I: two or more NoV positive samples per outbreak to identify the causative agent; B^I: 50% or more NoV positive samples per outbreak to identify the causative agent.

Table 5
Proportion of NoV negative samples from outbreaks tested NoV negative by RT-PCR

Diagnostic test	No. of negative samples in NoV negative outbreaks (%)		No. of negative outbreaks (%)	
	Criterium A^I ($n=41$)	Criterium B^I ($n=70$)	Criterium A^I ($n=8$)	Criterium B^I ($n=11$)
Dako kit	40 (98)	63 (90)	8 (100)	11 (100)
Ridascreen kit	39 (95)	62 (89)	8 (100)	11 (100)

A^I: two or more NoV positive samples per outbreak to identify the causative agent; B^I: 50% or more NoV positive samples per outbreak to identify the causative agent.

The Ridascreen kit does not discriminate between the genogroups. Only 1 out of 6 (17%) RT-PCR positive NoV genogroup I samples and 7 of 12 (58%) RT-PCR positive NoV genogroup II samples were positive by the Ridascreen kit. The genogroup IV sample was negative.

3.4. Diagnosis of outbreaks

Most NoV diagnostic tests are done as part of outbreak investigation. Different criteria were used to identify the causative agent of the outbreak, criterion A (two or more positive samples per outbreak to determine the causative agent of an outbreak (Richards et al., 2003)) and B (50% or more positive samples to determine the causative agent of an outbreak (Vinje and Koopmans, 1996)).

When criterion A was applied, there were 15 NoV positive outbreaks, based on RT-PCR analysis. Of these 15 outbreaks, only 8 (53%) and 9 (60%) were diagnosed as NoV outbreaks by the Dako kit and Ridascreen kit, respectively. These outbreaks consisted of 117 samples, of which 73 (62%), 30 (26%) and 35 (30%) were tested NoV positive by RT-PCR, the Dako kit and the Ridascreen kit, respectively. Applying a less stringent criterion (one or more ELISA positive samples per outbreak) resulted in 12/15 (75%) or 14/15 (93%) NoV positive outbreaks with the Dako kit and Ridascreen kit, respectively (Table 4). The outbreaks which were considered negative for NoV by RT-PCR contain a total 41 samples. Of these samples, 40 (98%) tested negative for NoV by RT-PCR and the Dako kit and 39 (95%) samples were negative for NoV by the Ridascreen kit (Table 5).

A total of 12 outbreaks positive for NoV were detected by RT-PCR when criterion B was applied. When this criterion for the ELISA kits was applied a total of five (42%) and four (33%) NoV positive outbreaks were detected for the Dako kit and the Ridascreen kit, respectively. These 12 NoV positive outbreaks contained 88 samples, of which 62 (70%), 24 (27%) and 29 (33%) were scored as NoV positive by RT-PCR, the Dako kit and the Ridascreen kit, respectively (Table 4). The negative outbreaks contain 70 samples, of which 58 (83%), 63 (90%) and 62 (89%) were tested NoV negative by RT-PCR, the Dako kit and the Ridascreen kit, respectively (Table 5). None of the RT-PCR NoV negative outbreaks were found NoV positive with any of the ELISA tests in both criterions.

4. Discussion

The high diversity, genetic and antigenic, of NoV poses problems for diagnosis.

At present, RT-PCR is the method used most widely for detection of NoVs in human fecal specimens, however, due to the high genetic diversity constant updating of primers (and probes) is required which adds to the complexity of RT-PCR as a diagnostic method. The detection of NoV in human stool specimens by ELISA is based on detection of NoV antigens and may thus be hampered by the antigenic diversity of NoV. Several reports that compared antigen detection to RT-PCR showed that ELISAs were easier to carry out but also less sensitive than the RT-PCR methods (Burton-MacLeod et al., 2004; Richards et al., 2003; Rabenau et al., 2003). The two ELISA kits tested in this study were clearly less sensitive than the RT-PCR in detecting NoV in fecal samples. In outbreaks tested positive for NoV by RT-PCR, the proportion of samples tested positive for NoV by the ELISA kits was less than half the proportion tested positive for NoV by RT-PCR, irrespective of the criteria chosen to define a NoV outbreak.

A sensitivity of 38% was found for the Dako kit, similar to a previous observation (reported sensitivities of 39% and 31% for the Dako kit in samples from NoV outbreaks found positive by RT-PCR; Burton-MacLeod et al., 2004 and Rabenau et al., 2003, respectively). A sensitivity and specificity of 36% and 88% were found for the Ridascreen kit, which is comparable to the Dako kit. Dimitriadis and Marshall (2005) reported a sensitivity and specificity of 71% and 47% for single specimen diagnosis, respectively, and an even higher sensitivity and specificity for outbreak diagnosis for the Ridascreen kit. However, based on

this study, it is assumed that at least 40% of the NoV outbreaks would not be diagnosed as NoV outbreaks using any of the two commercial ELISAs evaluated.

The most dominant type of NoV, at least in epidemic years in Europe and Australia, is GGII.4 (Kirkwood et al., 2005; Lopman et al., 2004). A high sensitivity for GGII.4 viruses could in daily practice lead to a relatively good detection of NoV outbreaks. However, in this study the sensitivity of both ELISA kits tested for NoV GGII.4 was not different from the overall sensitivity.

The overall specificity of the Dako kit was satisfactory, none of the confirmed Astrovirus or Rotavirus containing samples cross-reacted with the Dako kit. The specificity of the Ridascreen kit was less than that of the Dako kit and the Ridascreen kit showed unexplained cross-reactivity with one of the samples containing rotaviruses. When seeking broad detection, a feature that is important given the high level of genetic and antigenic diversity of NoVs, the Ridascreen assay was clearly less optimal since viruses belonging to six genotypes were not detected in this study. Remarkably, viruses of GGI.3 and GGI.5 were not detected by any of the ELISAs.

The Dako kit consists of separate tests for genogroup I and II, but discrimination between genogroups was poor, 6 out of 12 genogroup II samples reacted with the EIA genogroup I wells. The poor discrimination was observed previously, however, in that study, 7% of the genogroup I samples reacted with the EIA genogroup II wells (Burton-MacLeod et al., 2004). Also, the relevance of the ability to discriminate between genogroup I and II is unclear; there is no difference between the clinical pictures of NoV genogroup I or II, and typing to genogroup level is not sufficiently precise for molecular epidemiology studies (Koopmans et al., 2001).

In conclusion, because of its rapidity and simplicity the ELISA kits may be useful for screening of large numbers of clinical samples during outbreaks. The assays are not suitable for diagnosis of infection in individual patients, or for genotype assignment. Because of the low sensitivity, RT-PCR remains the "gold standard" for routine diagnosis of NoV in samples from patients with gastroenteritis.

Acknowledgements

This work was supported by the European Commission, DG Research Quality of Life Program, Fifth Framework (QLK1-CT-1999-00594), Sixth Framework (SP22-CT-2004-502571) and DG SANCO (2003213).

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