

Zeitschrift: Mitteilungen aus Lebensmitteluntersuchungen und Hygiene = Travaux de chimie alimentaire et d'hygiène
Band: 94 (2003)
Heft: 6

Artikel: Evaluation of a commercial ELISA kit for the detection of Norovirus antigens in human stool specimens
Autor: Christen, Andri / Fretz, Rainer / Tanner, Marcel
DOI: <https://doi.org/10.5169/seals-982011>

Nutzungsbedingungen

Die ETH-Bibliothek ist die Anbieterin der digitalisierten Zeitschriften. Sie besitzt keine Urheberrechte an den Zeitschriften und ist nicht verantwortlich für deren Inhalte. Die Rechte liegen in der Regel bei den Herausgebern beziehungsweise den externen Rechteinhabern. [Siehe Rechtliche Hinweise.](#)

Conditions d'utilisation

L'ETH Library est le fournisseur des revues numérisées. Elle ne détient aucun droit d'auteur sur les revues et n'est pas responsable de leur contenu. En règle générale, les droits sont détenus par les éditeurs ou les détenteurs de droits externes. [Voir Informations légales.](#)

Terms of use

The ETH Library is the provider of the digitised journals. It does not own any copyrights to the journals and is not responsible for their content. The rights usually lie with the publishers or the external rights holders. [See Legal notice.](#)

Download PDF: 08.11.2024

ETH-Bibliothek Zürich, E-Periodica, <https://www.e-periodica.ch>

Evaluation of a commercial ELISA kit for the detection of *Norovirus* antigens in human stool specimens

Andri Christen^{1,2}, Rainer Fretz^{1,2}, Marcel Tanner² and Paul Svoboda¹

¹Cantonal Laboratory Basel-Landschaft, Liestal, Switzerland

²Swiss Tropical Institute, Basel, Switzerland

Received 16 July 2003, accepted 5 November 2003

Introduction

Noroviruses (NVs; formerly “*Norwalk-like viruses*”) are forming the genus *Norovirus* within the family *Caliciviridae*. They are nonenveloped, single stranded RNA viruses. The NV genus is subdivided in two human pathogenic genogroups based on sequence information of genes encoding the viral RNA-dependent RNA polymerase and the capsid protein: Genogroup I with the Norwalk virus as the prototype strain, and Genogroup II with Snow Mountain and Hawaii virus (1).

NVs are mainly transmitted from person to person by the faecal-oral and aerosol route and constitute the most common cause of acute non-bacterial gastroenteritis in industrialised countries (2). Established surveillance systems from ten European countries found NVs to be responsible for >85 % of all nonbacterial outbreaks of gastroenteritis reported from 1995 to 2000 (3). The same survey found that in Denmark, England and Wales, Finland, France and Sweden, >95 % of nonbacterial outbreaks were attributed to NVs (3). In the United States, estimated 30.8 million (80 %) of 38.6 million cases of gastroenteritis are the result of viral infections (4). NVs are more and more the most verified agent for large outbreaks of diarrhea in hospitals (5, 6), nursing and residential homes (7–9) and other institutional settings.

NVs strains exhibit wide genetic diversity, and viruses of both genogroups I and II and different genotypes co-circulate simultaneously in any geographical region (10).

Despite numerous attempts NV infections have not been induced in experimental animals, nor have these viruses been propagated successfully in cell culture. Currently, the most widely used detection method for NVs in stool samples is the reverse transcription-polymerase chain reaction (RT-PCR) (12). Using several

primer pairs and sequence analysis of the PCR products, the RT-PCR has proven to be the most sensitive method for detection of NVs and is now regarded as the gold standard (5, 12). However, this method has several drawbacks: difficulties in extracting RNA from faecal samples, the presence of amplification inhibitors in the stool and the genetic diversity of NVs complicating the design of oligonucleotide primers detecting all genotypes in a single assay. Also the time needed to complete the analysis is a critical factor (10), since rapid identification of pathogens is essential for patient care and for a prompt and appropriate intervention to control and prevent outbreaks of disease (12).

Further molecular engineering techniques allowed the development of more rapid technique like enzyme-linked immunosorbent assays (ELISA) based on monoclonal antibodies to recombinant NV capsid proteins (13, 14). The enhancement of these techniques allowed the production of multivalent antibodies with wide-ranging reactivities, which are able to detect a broad range of NV genotypes within the two NV genogroups.

In this study we evaluated the kit IDEIA™ Norwalk-like Virus ELISA (DakoCytomation, Ely, UK), commercially available since 2002, for the detection of NV in stool samples. The assay functions with polyclonal and monoclonal antibodies specific for capsid proteins of NV genotypes within genogroups I and II.

Materials and Methods

Stool samples

In Switzerland clinical microbiology laboratories do not routinely screen for NVs and there is no obligation to report NV infections. For this reason, the Swiss Federal Office of Public Health (SFOPH) launched two epidemiological projects to clarify the situation of *Norovirus* infections in Switzerland: i) a case control study to identify the Swiss specific risk factors and the endemicity of the NV infections in the German speaking part of Switzerland; ii) a systematic investigation of NV outbreaks in Switzerland (15).

Overall, 39 stool samples from patients involved in the case control study described above, and from several NV outbreaks, were tested for NVs using the IDEIA™ Norwalk-like Virus ELISA from DakoCytomation. Over six months (June 2002–December 2002), 28 samples were collected from eight different outbreaks. 11 samples were recruited from the case control study (January 2002–November 2002). The 39 samples were previously tested by RT-PCR as NV positive (n=21) or negative (n=18). The RT-PCR used for the verification of NV in stool samples in this study is the standard method applied in the Cantonal Laboratories of Basel-Landschaft (centre of competence for the Epidemiology of NV) and of Solothurn and is published (16).

Additional 24 stool samples analysed by ELISA were previously tested by RT-PCR for NV (13 NV positive/11 NV negative) in the German Laboratory

Prof. Seelig & Colleagues. The majority of the Swiss-based stool samples were stored at 4°C and some of them were older than several weeks or months. Only six samples were a few days old and were stored at -20°C. The German-based stool samples were stored as indicated in the manual of the ELISA test for appropriate use: storage of specimens 2-8°C for three days prior to testing, for long-term storage freezing of samples by -20°C.

ELISA test procedure

The test uses genogroup I and genogroup II specific monoclonal antibodies, attached to the microtitre wells, in a solid-phase sandwich enzyme immunoassay. The assay was performed according to the manufacturer's instructions. The evaluation of the samples was done visually and photometrically.

Viral RNA extraction and RT-PCR

In order to avoid the often-described difficulties in isolating viruses from stool samples, a total of 100 µl of faeces were diluted 1:10 with potable water from the water tap. Samples were vortexed and centrifuged and 140 µl of the supernatant were lysed in 560 µl AVL-buffer (QIAGEN GmbH, Hilden, Germany). The RNA was extracted following the manufacturer's standard protocol for small volumes of the QIAmp® viral RNA mini kit (QIAGEN GmbH, Hilden, Germany).

Two RT-PCR systems described by *Beuret et al.* (16) with two different primer sets were used. The genogroup-specific NV RT-PCR system for the detection of NV genogroup II is based on degenerate primers located in highly conserved regions of the RNA polymerase. The predicted product size is 203 bp. The second NV-RT-PCR system is based on generic degenerate primers. The predicted product size is 213 bp. An internal positive control with known sequence identity and a negative control (tap water) were used, accompanying the analysed stool samples.

Statistical methods

Using the RT-PCR method as a reference method, the concordance between the RT-PCR method and the ELISA test was assessed by calculating the Kappa index. The concordance index Kappa is a measure of accordance with two microbiological test procedures. A Kappa of "zero" denotes no agreement and a Kappa of "one" denotes perfect agreement. The concordance of two different methods was defined as sufficient when the Kappa value is bigger than 0.81 (17).

Results

Nine Swiss and six German stool samples were positive for NVs by ELISA and RT-PCR. A further 15 Swiss and 11 German samples were negative in both assays. 15 Swiss and 7 German samples gave discrepant results, giving an agreement of 62% (Kappa=0.25) for the Swiss samples and 71% (Kappa=0.44) for the German samples comparing both NV detection methods (tables 1 and 2). For Swiss samples,

the ELISA had a sensitivity of 43 % and a specificity of 83 % when compared with the RT-PCR (table 1). German samples gave a sensitivity of 46 % and a specificity of 100 % (table 2). Two of three ELISA positive and RT-PCR negative Swiss stool samples (ID 16, 25, 29) were RT-PCR positive several months before this study. When the samples were tested positive only by RT-PCR with the generic primer set (Mon 431-Mon 434), the antigens in the ELISA were only detected by the genogroup I specific antibodies. The sequencing and the phylogenetic comparison of the Swiss samples show that the ELISA detected NV strains in the whole spectrum of genetic diversity of NV strains co-circulating in the German speaking part of Switzerland (figure 1). The NV strains detected by both primer sets or only by the generic primers have a sequence identity of 92.6 % to 95.1 % with NV/Miami Beach/326/1995/US (GenBank accession No AF414424). The NV strain detected in the sample with the ID 24 has a sequence identity of 90 % to NV/LittleRock/316/1994/US (AF414405) and 90 % to NV/Honolulu/219/1992/US (AF414403). Sequence analyses were performed using the software EMBOSS matcher 2.0u4. The ELISA detected NV strains in stool samples of four out of eight outbreaks with an average number of 2.8 samples per outbreak. There is no evidence that strains not detected by ELISA differed from strains that were detected by ELISA, because of the lack of a specific pattern in the phylogenetic distribution of the detectable and non-detectable NV isolates in the phylogenetic tree.

No further genetic information about the German stool samples was available.

Table 1
Statistics of the photometrical evaluation of the Swiss samples (ELISA)

		ELISA				
		+	-	Sum		
RT-PCR	+	9	12	21	Specificity	0.83
	-	3	15	18	Sensitivity	0.43
Sum		12	27	39	Relative trueness	0.62
					False positive	0.17
					False negative	0.57
					Concordance index Kappa	0.25

Table 2
Statistics of the photometrical evaluation of the German samples (ELISA)

		ELISA				
		+	-	Sum		
RT-PCR	+	6	7	13	Specificity	1.00
	-	0	11	11	Sensitivity	0.46
Sum		6	18	24	Relative trueness	0.71
					False positive	0.00
					False negative	0.54
					Concordance index Kappa	0.44

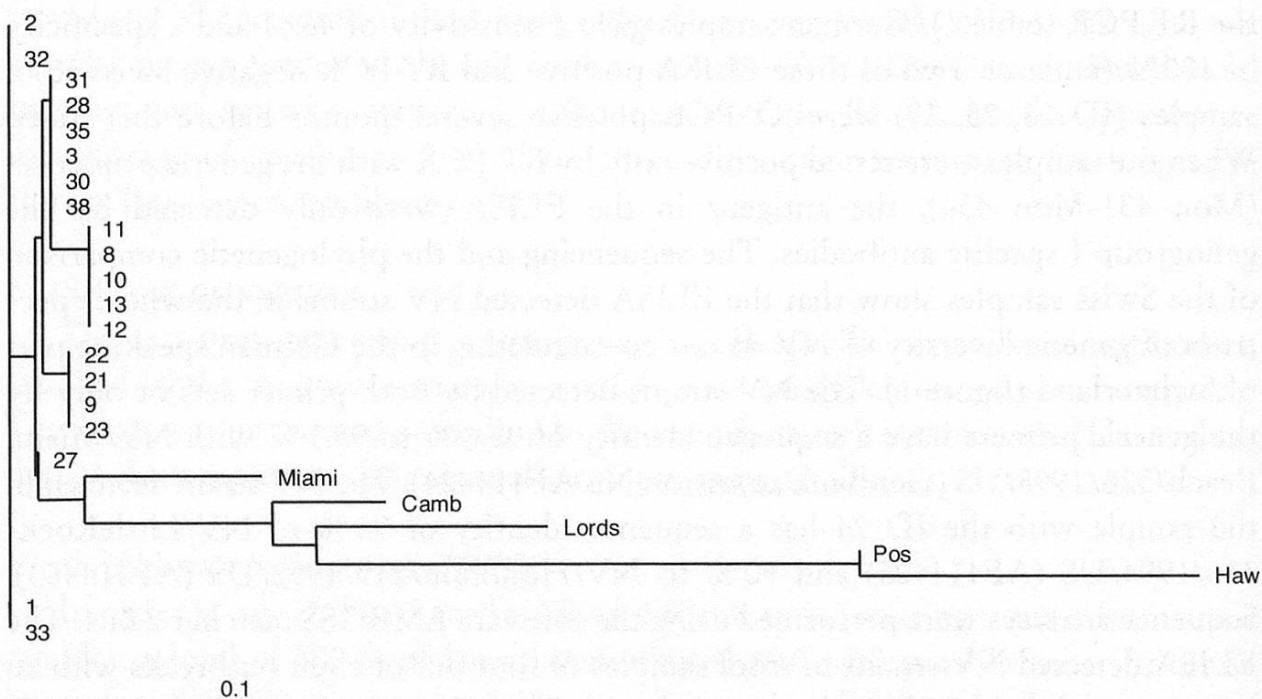


Figure 1 Phylogram of Kimura's distance using the neighbour joining method of analysis

Phylogram generated from RNA polymerase sequence information of Swiss *Norovirus* isolates (numbers), the used internal positive control (Pos) and sequences from GenBank (Haw: Hawaii Calicivirus HCU07611; Lords: Lordsdale virus X86557; Camb: Camberwell virus AF145896; Miami: Norwalk-like virus NLV/Miami Beach/326/1995/US AF414424). The internal positive control (Pos) has a 96% sequence identity with NV/Westover/302/1994/US AF414418. Software used to create NJ was ClustalX 1.81, PAUP 4.0b10.

Discussion

Up to now, analyses of acute gastroenteritis outbreaks in Switzerland were performed by using molecular amplification methods (RT-PCR). Although PCR-analyses are expensive and time consuming, they have been proclaimed as the "gold standard method" (12). The demand for a simplified, rapid and less expensive method for detecting NV in stool samples is therefore increasing. New ELISA kits offer a screening method for the detection of NVs, as antigen capture assays should be broadly reactive to overcome non-specific reactivity associated with the use of stool material in enzyme immunoassays.

An evaluation of the same ELISA kit was done in England by A.F. Richards (10) and shows that a median of 2 (range 0–6) samples were positive by ELISA in RT-PCR positive outbreaks. This demonstrates the need to collect samples from a minimum of six affected individuals when undertaking an outbreak investigation by using ELISA. In this study six samples were available from only one outbreak out

of eight. The average number of samples per outbreak was 2.8. This also represents the current sample average available from outbreaks in the German speaking part of Switzerland during outbreak investigations. The same English evaluation revealed an overall sensitivity and specificity of 55.5 % and 98.3 % respectively for the ELISA (10). The differences in the sensitivities between the Swiss and the English evaluation (approx. 45 % and 55.5 % respectively) may be explained by the differences within the used RT-PCR methods and by variation in sample size. To respond to a possible influence of inappropriate storing of the stool samples, two set of samples were used. The majority of the Swiss-based stool samples were stored at 4 °C and some of them were older than several weeks or months. In contrast, the German-based stool samples were stored as indicated in the manual of the ELISA test for appropriate use (storage of specimens 2–8 °C for three days prior to testing, for long-term storage freezing of samples by –20 °C). The small differences between the sensitivities of the Swiss and of the German samples (43 % and 46 % respectively) indicate that the storage may play a minor role within this study. Due to its low sensitivity of approx. 45 %, the ELISA kit is unsuitable for the Swiss Cantonal Laboratories and for medical diagnostic laboratories, which mostly investigate sporadic NV cases with a small number of samples provided. The ELISA kit could be useful as a NV screening method for investigating outbreaks of gastroenteritis in institutions missing specialised molecular detection methods but having a high number of possible NV cases, for example hospitals. Samples from outbreaks which are negative by ELISA should be analysed by RT-PCR to detect NV non-reactive in the ELISA essay. The sensitivity and the specificity of the ELISA have to be increased if possible in order to make it more interesting for a wide application.

Acknowledgement

The authors thank Ms. B. Fallowfield for the critical review of the manuscript; the Swiss medical diagnostic laboratory Viollier (especially L. Herrmann) and the Laboratory Prof. Seelig & Colleagues (especially Dr. B. Miller) for the cooperation and for providing the stool samples; and J. Grimbichler, Cantonal Laboratory Basel-Landschaft, for advice on the analyses.

Summary

Noroviruses (NV, formerly “*Norwalk-like viruses*”) are responsible for >85 % of all nonbacterial outbreaks of gastroenteritis in Europe and are typically detected in stool by reverse transcription polymerase chain reaction (RT-PCR) or electron microscopy. More rapid and less expensive methods like the ELISA (enzyme-linked immunosorbent assay) for detection of NVs in stool samples are now commercially available. The ELISA kit IDEIA™ (DakoCytomation, Ely, UK) for the detection of NV antigens in stool samples and the simultaneous determination of the NV genogroup was evaluated. The performance of the ELISA was compared with an RT-PCR by testing stool samples collected from patients involved in NV outbreaks

of gastroenteritis from Switzerland and Germany. We tested 39 Swiss stool samples and found for the ELISA a sensitivity and specificity of 43 % and 83 % respectively. When tested with German stool samples, the ELISA had a sensitivity and specificity of 46 % and 100 % respectively. Due to its low sensitivity, the ELISA kit is unsuitable for the Swiss Cantonal Laboratories and for medical diagnostic laboratories, which mostly investigate sporadic NV cases with a small number of samples provided. The ELISA kit could be useful as a NV screening method for investigating outbreaks of gastroenteritis in institutions missing specialised molecular detection methods but having a high number of possible NV cases, for example hospitals.

Résumé

En Europe, les Norovirus (NV, autrefois «*Norwalk-like viruses*») sont responsables de >85 % des épidémies de gastroenterites non bactériennes. Leur détection dans des échantillons de selles se fait en moyen de la reverse transcription polymérase chain reaction (RT-PCR) ou la microscopie électronique. De nouvelles méthodes, plus rapides et moins chères, dont un test ELISA (enzyme-linked immunosorbent assay) qui permet également la détection des NV dans les selles, sont maintenant disponibles. Le kit ELISA IDEIA™ (DakoCytomation, Ely, UK), développé pour la détection d'antigènes de NVs dans des échantillons de selles et pour la détermination simultanée de leurs génogroupes, a été évalué. Des échantillons de selles ont été examinés avec ce test ELISA et la performance a été comparée à celle d'une analyse par RT-PCR. Les échantillons provenaient de patients impliqués dans des foyers épidémiques de gastroenterite causés par des NV en Suisse et en Allemagne. Au total, 39 échantillons de selles suisses ont été examinés avec les deux méthodes. Par rapport à la RT-PCR, les analyses avec le test ELISA ont montré une sensibilité de 43 % et une spécificité de 83 %. Parmi les échantillons allemands, la sensibilité s'élevait à 46 % et la spécificité à 100 %. En raison de son faible sensibilité, le kit ELISA ne se prête pas à la détection de NV pour les laboratoires cantonaux suisses ou les laboratoires de diagnostic, lorsqu'il s'agit d'examiner des cas sporadiques avec un petit nombre d'échantillons. Par contre, en cas d'investigations épidémiologiques dans des institutions ne disposant pas de méthodes de détection moléculaire, le kit ELISA peut s'avérer utile pour la détection des NV parmi un nombre élevé d'échantillons, par exemple lors d'investigations d'un foyer épidémique dans un hôpital.

Zusammenfassung

Die Noroviren (NV, ehemals «*Norwalk-like Viren*») sind für >85 % aller nicht-bakteriellen Gastroenteritis-Epidemien in Europa verantwortlich und werden mittels der Reversen Polymerasenkettenreaktion (RT-PCR) oder mittels Elektronenmikroskopie in Patientenstuhlproben nachgewiesen. Schnellere und kostengünstigere Methoden wie kommerzielle ELISA (enzyme-linked immunosorbent assay) für den Noroviren-Nachweis sind mittlerweile erhältlich. Der ELISA Kit

IDEIA™ (DakoCytomation, Ely, UK) für den Nachweis von NV-Antigenen in Stuhlproben und für die Bestimmung der NV-Genogruppe wurde evaluiert. Die Detektionsleistungen des ELISA versus RT-PCR wurden verglichen, indem Patientenstuhlproben von Gastroenteritis-Epidemien aus der Schweiz und Deutschland untersucht wurden. 39 Schweizer Stuhlproben wurden analysiert und der ELISA ergab eine Sensitivität von 43 % und eine Spezifität von 83 %. Weiter wies der ELISA-Test bei den untersuchten deutschen Stuhlproben eine Sensitivität von 46 % und eine Spezifität von 100 % auf. Aufgrund der niedrigen Sensitivität ist der ELISA Kit ungeeignet für die schweizerischen kantonalen Laboratorien und für medizinische Diagnostiklaboratorien, da letztere vor allem sporadische NV-Fälle untersuchen wo das Probenvolumen gering ist. Der ELISA Kit könnte jedoch innerhalb von Institutionen von Nutzen sein, welche über keine spezialisierten molekularen Nachweismethoden verfügen, aber eine hohe Anzahl von möglichen NV-Fällen aufweisen, wie zum Beispiel Spitäler.

Key words

Norovirus, gastroenteritis, detection, ELISA, RT-PCR

References

- 1 Green J., Vinje J., Gallimore C.I., Koopmans M., Hale A. and Brown D.W.: Capsid protein diversity among Norwalk-like viruses. *Virus Genes* 20, 227–236 (2000)
- 2 Guerrant R.L., Hughes J.M., Lima N.L. and Crane J.: Diarrhea in developed and developing countries: magnitude, special settings, and etiologies. *Rev Infect Dis* 12 Suppl 1, 41–50 (1990)
- 3 Lopman B.A., Reacher M.H., Van Duynhoven Y., Hanon F.-X., Brown D. and Koopmans M.: Viral gastroenteritis outbreaks in Europe, 1995–2000. *Emerg Infect Dis* 9, 90–96 (2003)
- 4 Mead P.S., Slutsker L., Dietz V., McCaig L.F., Bresee J.S., Shapiro C., Griffin P.M. and Tauxe R.V.: Food-related illness and death in the United States. *Emerg Infect Dis* 5, 607–625 (1999)
- 5 Green S.M., Lambden P.R., Deng Y., Lowes J.A., Lineham S., Bushell J., Rogers J., Caul E.O., Ashley C.R. and Clarke I.N.: Polymerase chain reaction detection of small round-structured viruses from two related hospital outbreaks of gastroenteritis using inosine-containing primers. *J Med Virol* 45, 197–202 (1995)
- 6 Green J., Wright P.A., Gallimore C.I., Mitchell O., Morgan-Capner P. and Brown D.W.G.: The role of environmental contamination with small round structured viruses in a hospital outbreak investigated by reverse transcriptase polymerase chain reaction assay. *J Hosp Infect* 39, 39–45 (1998)
- 7 Green K.Y., Belliot G., Taylor J.L., Valdesuso J., Lew J.F., Kapikian A.Z. and Lin F.Y.: A predominant role for Norwalk-like viruses as agents of epidemic gastroenteritis in Maryland nursing homes for the elderly. *J Infect Dis* 185, 133–146 (2002)
- 8 Jiang X., Turf E., Hu E., Barrett E., Dai X.M., Monroe S., Humphrey C., Pickering L.K. and Matson D.O.: Outbreaks of gastroenteritis in elderly nursing homes and retirement facilities associated with human caliciviruses. *J Med Virol* 50, 335–341 (1996)
- 9 Kaplan J.E., Schonberger L.B., Varano G., Jackman N., Bied J. and Gary G.W.: An outbreak of acute nonbacterial gastroenteritis in a nursing home. Demonstration of person-to-person transmission by temporal clustering of cases. *Am J Epidemiol* 116, 940–958 (1982)

- 10 Richards A.F., Lopman B., Gunn A., Curry A., Ellis D., Cotterill H., Ratcliffe S., Jenkins M., Appleton H., Gallimore C.I., Gray J.J. and Brown D.W.G.: Evaluation of commercial ELISA for detecting Norwalk-like virus antigen faeces. *J Clin Virology* **26**, 109–115 (2003)
- 11 Glass R.I., Noel J., Ando T., Fankhauser R., Belliot G., Mounts A., Parashar U.D., Bresee J.S., and Monroe S.S.: The epidemiology of enteric caliciviruses from humans: a reassessment using new diagnostics. *J Infect Dis* **181**, 254–261 (2000)
- 12 Green S.R., Moe C.L., Jaykus L.-A., Cronin M., Grosso L. and van Aarle P.: Evaluation of the NucliSens® Basic Kit assay for detection of Norwalk virus RNA in stool specimens. *J Virol Methods* **108**, 123–131 (2003)
- 13 Brinker J.P., Blacklow N.R., Estes M.K., Moe C.L., Schwab K.J. and Herrmann J.E.: Detection of Norwalk virus and other genogroup 1 human caliciviruses by a monoclonal antibody, recombinant-antigen-based immunoglobulin M capture enzyme immunoassay. *J Clin Microbiol* **36**, 1064–1069 (1998)
- 14 Herrmann J.E., Blacklow N.R., Matsui S.M., Lewis T.L., Estes M.K., Ball J.M. and Brinker J.P.: Monoclonal antibodies for detection of Norwalk virus antigen in stools. *J Clin Microbiol* **33**, 2511–2513 (1995)
- 15 Fretz R.: Annual report 2002, Cantonal Laboratory Basel-Landschaft, January 13 (2003)
- 16 Beuret C.: A simple method for isolation of enteric viruses (Noroviruses and Enteroviruses) in water. *J Virol Methods* **107**, 1–8 (2003)
- 17 Hübner P., Gautsch S. und Jemmi T.: In house-Validierung mikrobiologischer Prüfverfahren. *Mitt Gebiete Lebensm Hyg* **93**, 118–139 (2002)

Address of correspondent: Dr Paul Svoboda, Cantonal Laboratory Basel-Landschaft, CH-4410 Liestal, paul.svoboda@vsd.bl.ch