RIDASCREEN® Norovirus

3rd Generation

Article no.: C1401

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1. Intended use

For *in vitro* diagnostic use. RIDASCREEN® Norovirus 3rd Generation is an enzyme immune-assay for the qualitative identification of norovirus genogroups I and II in human stool samples.

2. Summary and explanation of the test

Noroviruses are a significant cause of gastroenteritis worldwide, with an estimated 23 million cases per year in the USA (1, 2). They are often found to be involved when infection breaks out in institutions and communities like care facilities, hospitals, child daycare, and prisons, or on cruise ships (3, 4, 5). Although the outbreaks of norovirus infection can be a considerable burden to public health, they are more often reported as outbreaks of bacterial infection (6).
The gastroenteritis caused by a norovirus presents strong nausea, forceful vomiting, and severe diarrhea. There is an incubation period of 6 to 48 hours, after which the symptoms may persist for 12 to 60 hours. The infectious dose of only 100 virus particles is extremely low, which provides the condition for this virus to spread very effectively from person to person. Because the virus is excreted both in vomit and stool, the formation of aerosol droplets carrying the virus makes the aerogenic route of transmission as significant as the fecal-oral transmission. That is evident in the often very rapid spread of the infection in social community environments.
With few exceptions, the virus is excreted for a period of about two weeks, which creates additional risk of further contagion. Norovirus reinfection is possible, not in the least because the pronounced variability does not allow a comprehensive immunity.
The usual diagnostic methods of stool sample analysis are limited to electron microscopy or mainly to molecular identification of the genome by polymerase chain reaction (PCR). Because these methods are relatively demanding, also requiring special expertise and specific laboratory-technical equipment, a more simple and faster screening test is needed. Already in its third generation of continued development, this ELISA is a highly relevant response to that challenge. The RIDASCREEN® Norovirus ELISA with monoclonal antibodies enables a very specific and highly sensitive identification of both norovirus genogroups.

3. Test principle

The RIDASCREEN® Norovirus 3rd Generation Test employs specific monoclonal antibodies in a sandwich-type method. The well surface of the microwell plate is coated with specific antibodies to the antigens of several different genotypes.
A pipette is used to place a suspension of the stool sample to be examined as well as the controls in the well of the microwell plate together with biotinylated monoclonal anti-norovirus antibodies (Conjugate 1) at room temperature (20 – 25 °C) for incubation. After a wash step, streptavidin poly-peroxidase conjugate (Conjugate 2) is added and it is incubated again at room temperature (20 – 25 °C). With the presence of noroviruses in a stool sample, a sandwich complex will form which consists of immobilized antibodies, the norovirus antigens, and the antibodies conjugated with the biotin-streptavidin-peroxidase complex. Another wash step removes the unattached streptavidin poly-peroxidase conjugate. After adding the substrate, the

attached enzyme changes the colour of the previously colourless solution in the wells of the microwell plate to blue if the test is positive. Addition of a stop reagent changes the color from blue to yellow. The extinction is proportional to the concentration of noroviruses found in the specimen.

4. Reagents provided

The reagents in the kit are sufficient for 96 determinations.

Plate	96	Microwell plate, 12 microwell strips (which can be divided) in the strip holder; coated with monoclonal anti-norovirus antibodies
Diluent | 1	100 ml	Sample dilution buffer, protein-buffered NaCl solution, ready to use, blue colored
Wash	100 ml	Wash buffer, phosphate-buffered NaCI solution (concentrated 10-fold); contains 0.1% thimerosal
Control | +	2 ml	Positive control (inactivated *Norovirus* capsid protein); ready for use
Control | -	2 ml	Negative control (sample dilution buffer); ready for use
Conjugate | 1	13 ml	Biotin-conjugated anti-norovirus antibodies in stabilized protein solution; ready to use; blue color
Conjugate | 2	13 ml	Streptavidin poly-peroxidase conjugate in stabilized protein solution; ready for use; orange color
Substrate	13 ml	Hydrogen peroxide/TMB; ready for use
Stop	12 ml	Stop reagent; 1 N sulphuric acid; ready for use

5. Reagents and their storage

All reagents must be stored at 2 – 8 °C and can be used until the expiry date printed on the label. Providing the diluted wash buffer is stored at 2 – 8 °C, it can be used for a maximum of 4 weeks. Microbial contamination must be prevented. After the expiry date, the quality guarantee is no longer valid.

The aluminium bag must be opened with scissors in such a way that the clip seal is not torn off. Any microwell strips which are not required must be returned to the aluminium bag and stored immediately at 2 – 8 °C.

The colorless substrate must be protected from direct light to prevent it from decomposing or turning blue due to auto-oxidation. Once the substrate has turned blue, it must not be used.

6. Additional necessary reagents – and necessary equipment

6.1. Reagents

– Distilled or deionized water

6.2. Equipment

– Test tubes
– Disposable pipettes (Article no.: Z0001)
– Vortex mixer (optional, see 9.3.)
– Micropipette for 50 – 100 µl and 1 ml volumes
– Measuring cylinder (1000 ml)
– Timer
– Washing device for microwell plates or multichannel pipettes (300 µl)
– Photometer for microwell plates (450 nm and reference filter 620 – 650 nm)
– Filter paper (laboratory towels)
– Waste container with a 0.5 % hypochlorite solution

7. Precaution for users

For *in vitro* diagnostic only.

This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories must be followed. Always adhere strictly to the user instructions for this test.
Specimens or reagents must not be pipetted by mouth, and contact with injured skin or mucous membranes must be prevented. Wear personal safety gear (suitable gloves, laboratory coat, safety glasses) when handling the specimens, and wash hands after finishing the test. Do not smoke, eat, or drink in areas where samples are being processed.
For more details, refer to Material Safety Data Sheets (MSDS) at www.r-biopharm.com.
The positive control in the kit contains inactivated Norovirus capsid protein. It must be treated as potentially infectious material and handled in accordance with the national safety regulations, just like the patient sample.
The wash buffer contains 0.1 % thimerosal as preservative. This substance must not be allowed to come into contact with skin or mucous membranes.
Ensure the proper and responsible disposal of all reagents and materials after their use. For disposal, please adhere to national regulations.

8. Specimen collection and storage

Stool samples must be taken as soon as possible within three days after occurrence of the initial symptoms of diarrhea. Until it is used, store the test material at 2 – 8 °C. If the material cannot be used for a test within three days, we recommend storage at -20 °C or colder. Avoid freezing and thawing the specimen repeatedly. After diluting a stool sample in sample dilution buffer 1:11, it can be stored at 4 °C℃ for use within seven days.
Stool samples and rectal smears should not be collected in transport containers which contain

transport media with preservatives, animal sera, metal ions, oxidizing agents, or detergents since these may interfere with the RIDASCREEN® Norovirus 3rd Generation Test.
If rectal smears are used, make sure that the volume of stool material is sufficient (approx. 100 mg) for the test.
Contact tracing should include stool samples taken from contact persons who do not exhibit clinical symptoms, in order to identify asymptomatic carriers.

9. Test procedures

9.1. General information

All reagents and the microwell Plate must be brought to room temperature (20 – 25 °C) before use. The microwell strips must not be removed from the aluminium bag until they have reached room temperature. The reagents must be thoroughly mixed immediately before use. After use, the microwell strips (placed in sealed bags) and the reagents must be stored again at 2 – 8 °C. Once used, the microwell strips must not be used again. The reagents and microwell strips must not be used if the packaging is damaged or the vials are leaking.
In order to prevent cross contamination, the samples must be prevented from coming into direct contact with the kit components.
The test must not be carried out in direct sunlight. We recommend covering the microwell plate or placing plastic wrap over it to prevent evaporation losses.

9.2. Preparing the wash buffer

Mix 1 part wash buffer concentrate Wash with 9 parts distilled water. Any crystals present in the concentrate must be dissolved beforehand by warming in a water bath at 37 °C.

9.3 Preparing the samples

Fill a labelled test tube with 1 ml RIDASCREEN® sample dilution buffer Diluent | 1. Use a disposable pipette (article no. Z0001) to aspirate a sample of thin stool (approx. 100 µl) to just above the second marking and add to buffer in the test tube to make a suspension. To make a suspension with a solid stool sample, add an equivalent amount (approx. 50 – 100 mg) with a spatula or disposable inoculation loop.
Homogenize the stool suspension by aspiration into and ejection from a disposable pipette or, alternatively, blend in a Vortex mixer. Let the suspension stand a short period of time (10 minutes) for the coarse stool particles to settle, and this clarified supernatant of the stool suspension can be used directly in the test. If the test procedure is carried out in an automated ELISA system, the supernatant must be particle-free. In this case, it is advisable to centrifuge the sample at 2500 G for 5 minutes.

Note:

Stool samples diluted in Diluent | 1 can be tested in all RIDASCREEN® ELISA for which Diluent | 1 is used.

9.4. First incubation

After inserting a sufficient number of wells in the strip holder, add 100 µl of the positive Control | + , the negative Control | - or the stool sample suspension to the wells. Subsequently add 100 µl of the biotin-conjugated antibody Conjugate | 1 and blend (by tapping lightly on the side of the plate); then incubate for 60 minutes at room temperature (20 – 25 °C).

9.5. Washing

Careful washing is important in order to achieve the correct results and should therefore proceed strictly according to the instructions. The incubated substance in the wells must be emptied into a waste container for disposal in accordance with local regulations. After this, knock out the plate onto absorbent paper in order to remove the residual moisture. Then wash the plate five times using 300 µl wash buffer each time. Make sure that the wells are emptied completely by knocking them out after each wash on a part of the absorbent paper which is still dry and unused.
If you use a microplate washer or fully automated ELISA, make sure that the machine is correctly adjusted; request settings from the manufacturer, if necessary. Appliances delivered by R-Biopharm are already programmed with validated settings and work protocols. To avoid blocking the wash needles, only particle-free stool suspensions should be dispensed (see Item 9.3., Preparing the samples). Also make sure that all of the liquid is aspirated during each wash step.

9.6. Second incubation

Use a pipette to fill 100 µl streptavidin poly-peroxidase conjugate Conjugate | 2 into the wells, then incubate for 30 minutes at room temperature (20 – 25 °C).

9.7. Washing

Wash as described in Item 9.5.

9.8. Third incubation

Fill all wells with 100 µl substrate Substrate. Then incubate the plate for 15 minutes in darkness at room temperature (20 – 25°C). Subsequently fill all wells with 50 µl stop reagent Stop in order to stop the reaction. After blending cautiously by tapping lightly on the side of the plate, measure the extinction at 450 nm (optional: 450/620 nm). Adjust the zero point in the air that is without the microwell plate.
Note:
High-positive patient samples may cause black-colored precipitates of the substrate.

10. Quality control – indications of reagent expiry

For quality control purposes, positive and negative controls must be used each time the test is carried out, to ensure that the reagents are stable and that the test is conducted correctly. The

test has been carried out correctly if the extinction rate (O.D.) for the negative control is less than 0.2 at 450 nm (less than 0.160 at 450/620 nm) and the measured value for the positive control is greater than 0.8 at 450 nm or at 450/620 nm. A value greater than 0.2 (0.160) for the negative control may indicate that washing was insufficient. Deviation from the required values, just like a turbid or blue coloring of the colorless substrate before it is filled into the wells, may indicate that the reagents have expired.
If the stipulated values are not met, the following points must be checked before repeating the test:

- Expiry date of the reagents used
- Functionality of the equipment being used (e.g. calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks – a substrate solution which has turned blue must not be used.

If the conditions are still not fulfilled after repeating the test, please consult the manufacturer or your local R-Biopharm distributor.

11. Assessment and interpretation

11.1. Calculating the cut-off

In order to establish the cut-off, 0.15 extinction units are added to the measured extinction for the negative control.

Cut-off = extinction for the negative control + 0.15

11.2. Test results

Assessment of the specimen is positive if the extinction rate is more than 10 % higher than the calculated cut-off value.
Assessment of the specimen is marginal if the extinction rate ranges from 10 % less to 10 % greater than the cut-off value. If the repeat examination with a fresh stool sample again falls within the gray zone, assessment of the sample is negative.
Samples with extinctions more than 10 % below the calculated cut-off must be considered negative.

12. Limitations of the method

The RIDASCREEN® Norovirus 3rd Generation Test identifies antigens of the norovirus in stool samples, detecting both genogroups (I and II) that cause disease in humans. Not all genotypes and related sub-types were tested, but nearly all of the genotypes known from gastroenteritis outbreaks up to this time can be detected, as long as the viral load in the sample does not range below the detection limit of the ELISA at the time of sample taking. It is not possible to associate the determined level of extinction to the occurrence or severity of clinical symptoms. The results obtained must always be interpreted in combination with the clinical picture. It is

possible for asymptomatic persons to excrete the virus, and they can be found with this ELISA (contact tracing). The available virus load is always decisive.
A positive result does not rule out the presence of other infectious pathogens. The literature sufficiently describes double and multiple infections with potential gastroenteritis pathogens, and they can be diagnosed by differential methods. In many instances, the clinical signs and symptoms are more pronounced than those with monocausal origins.
A negative result does not rule out the possibility of norovirus infection. This may be explained by intermittent excretion of the virus, sample taking at an ill-suited point in time (see Item 8 "Specimen collection and storage"), a minimal viral load, or by inappropriate handling of the specimen. If the patient anamnesis supports a suspicion of norovirus infection, the examination should be repeated with another stool sample.
A borderline result may be explained by non-homogeneous distribution of viruses in the stool sample, viral load on the borderline for ELISA at the start, or insufficient washing of the microwells, or it may indicate that the infection is in regression. In this case, examination should either be repeated with a second suspension from the same sample or another stool sample should be requested.
The RIDASCREEN® Norovirus ELISA is not validated for meconium samples at this time, so results of those assays must be interpreted with caution. A variety of infant care products such as creams, oils and ointments which may transfer from the diaper to the stool specimen on sample taking have been used to spike norovirus-negative stool samples and were not found to affect the results of analysis. Neither Norovirus-positive stool samples were found to be affected negatively when tested with the brand-name products.

13. Performance characteristics

13.1. Test quality

A validation study with RIDASCREEN® Norovirus ELISA 3rd Generation involved 315 samples that had been examined before in a german routine laboratory. The prevalence for Norovirus was around 6.3 %. The results of that sample panel have been summarized in Table 1.

Table 1: Correlation between RIDASCREEN® Norovirus ELISA 3rd Generation and the routine diagnostic procedure in a German lab.

		Routine method	
		+	**-**
RIDASCREEN® Norovirus ELISA 3rd Generation	**+**	14	6
	-	6	289

Positive Concordance:	70,0 %
Negative Concordance:	98,0 %
Prevalence:	6,3 %

13.2. Cross reactivity

A variety of pathogenic microorganisms from the intestinal tract were examined with the RIDASCREEN® Norovirus ELISA. These studies were conducted with undiluted bacteria or virus suspensions shown to have concentrations of 10^6 to 10^9 organisms per ml. The results of that study are summarized in Table 2.

Table 2: Cross reactivity with pathogenic microorganisms

Organism	**Origin**	**[OD 450/620]**
Adenovirus	Culture supernatant	0.010
Aeromonas hydrophila	Culture	0.007
Astrovirus	Culture supernatant	0.012
Bacillus cereus	Culture	0.007
Bacteroides fragilis	Culture	0.025
Campylobacter coli	Culture	0.004
Campylobacter jejuni	Culture	0.005
Candida albicans	Culture	0.004
Citrobacter freundii	Culture	0.002
Clostridium difficile	Culture	0.002
Clostridium perfringens	Culture	0.002
Clostridium sordellii	Culture	0.003
E. coli (O157:H7)	Culture	0.000
E. coli (O26:H-)	Culture	0.004
E. coli (O6)	Culture	0.003
Enterobacter cloacae	Culture	0.002
Enterococcus faecalis	Culture	0.007
Klebsiella oxytoca	Culture	0.008
Proteus vulgaris	Culture	0.008
Pseudomonas aeruginosa	Culture	0.009
Rotavirus	Culture supernatant	0.010
Salmonella enteritidis	Culture	0.007
Salmonella typhimurium	Culture	0.007
Serratia liquefaciens	Culture	0.005
Shigella flexneri	Culture	0.007
Staphylococcus aureus	Culture	0.007
Staphylococcus epidermidis	Culture	0.049
Vibrio parahaemolyticus	Culture	0.009
Yersinia enterocolitica	Culture	0.008

13.3. Precision:

The reproducibility of RIDASCREEN® Norovirus ELISA was tested with six references representting the complete measurement range from weak to high positive. To determine the intra-assay reproducibility, 40 replicates of these references were assayed. The mean values and the variation coefficient (CV) were determined for three lots of the kits. For the inter-assay reproducibility, references from ten different working days were assayed in duplicates, with two runs per day. The measurements were determined by three technicians for three lots of the kits. The inter-lot reproducibility was determined for all three lots of the kits. As expected, Reference 6 results were negative in all of the analyses. The results of that study are shown in Table 3.

Table 3: Precision of the RIDASCREEN® Norovirus ELISA

Reference Mean value / CV (%)		**Intra-assay**			**Inter-assay**			**Inter-lot**
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1-3
1	MV [OD 450/620]	2.295	2.254	2.141	2.090	2.148	2.109	2.116
	CV (%)	5.86%	5.00%	5.75%	13.08%	10.23%	13.40%	12.29%
2	MV [OD 450/620]	1.524	1.617	1.482	1.499	1.574	1.414	1.496
	CV (%)	5.79%	5.48%	4.43%	13.23%	16.24%	18.10%	16.47%
3	MV [OD 450/620]	1.126	1.193	0.738	1.168	1.199	1.189	1.185
	CV (%)	6.19%	6.46%	7.06%	11.77%	13.61%	20.32%	15.70%
4	MV [OD 450/620]	0.578	0.655	0.577	0.649	0.666	0.586	0.634
	CV (%)	8.46%	5.46%	10.09%	16.22%	13.16%	19.87%	17.33%
5	MV [OD 450/620]	0.315	0.387	0.335	0.318	0.325	0.301	0.315
	CV (%)	5.80%	7.64%	8.02%	18.82%	17.17%	24.12%	20.07%
6	MV [OD 450/620]	0.009	0.022	0.036	0.019	0.017	0.011	0.016
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a

13.4 Analytical sensitivity

The analytic sensitivity of the RIDASCREEN® Norovirus ELISA was determined for two native stool samples (one each for the genogroups I and II), where the samples had previously been assayed for norovirus concentrations by electron microscope (EM) as well as real-time RT-PCR. To obtain a suitable virus concentration for the EM and PCR examinations, both samples were initially pre-diluted to 20% (1:5). Each pre-diluted sample was used to produce six more dilutions (series of 1 to powers of10). These six dilution steps were examined by way of the

RIDASCREEN® Norovirus ELISA (in triple measurements) and by EM and real-time RT-PCR. Concentrations in the individual dilutions were then calculated for the microscopic count and for the PCR measurement. The limit of detection (LoD) was determined at the concentration where the ELISA no longer delivered a positive result and it was denoted either as the RNA copies per gram of stool (PCR) or the particles per gram of stool (EM).

In the RIDASCREEN® Norovirus Test, the LoD for genogroup I (GI) is 1.51 x 10^6 RNA copies per ml of stool (1.18 x 10^7 particles per ml of stool) and LoD for genogroup II (GII) is 1.99 x 10^6 RNA copies per ml of stool. For the latter GII dilution step, the number of particles could no longer be determined by electron microscope, so this value was calculated out of the dilution steps measured before this point. The result of that calculation was 1.22 x 10^6 particles per ml of stool for GII (see Table 4, left column).

Based on the calculated LoD values per ml of stool, the limits of detection (LoD) were determined for concentrations of the analytes in the reaction mix (1:11 dilution of the sample). For GI, these results were 1.51 x 10^5 RNA copies per ml reaction mix and 1.18 x 10^6 particles per ml reaction mix. For GII, these results were 1.99 x 10^5 RNA copies per ml reaction mix and 1.22 x 10^5 particles per ml reaction mix (see Table 4, right side). All of the results are summarized in Table 4.

Table 4: LoD with RIDASCREEN® Norovirus

Genogroups	**In serial dilution**		**In reaction mix (1 part sample (from serial dilution + 10 parts Diluent 1)**	
	real-time RT-PCR [copies/ml]	EM [particles/ml]	real-time RT-PCR [copies/ml]	EM [particles/ml]
GGI	1.51 x 10^{6*}	1.18 x 10^{7*}	1.51 x 10^{5**}	1.18 x 10^{6**}
GGII	1.99 x 10^{6*}	1.22 x 10^{6*}	1.99 x 10^{5**}	1.22 x 10^{5**}

*Calculation based on the last dilution with a positive result in EM or PCR

**Calculated concentrations in assay-specific dilution in Diluent 1

14. Interfering substances

The following list of substances showed no effects on the test results when they were blended into norovirus positive and norovirus negative stool samples in the described concentrations**:**
barium sulfate (5% w/w), loperamide (antidiarrheal drug; 5% w/w), Pepto-Bismol (antidiarrheal drug, 5% v/w), mucins (5% w/w), cyclamate (artificial sweetener, 5% v/w), human blood (5% v/w), stearic acid and palmitic acid mixture (1:1, 40% w/w), metronidazole (0.5% solution; 5% v/w), diclofenac (0.00263% v/w).

Appendix

Test specific symbols:

Plate	Microwell plate
Diluent | 1	Sample dilution buffer
Wash	Wash buffer
Control | +	Positive control
Control | -	Negative control
Conjugate | 1	Conjugate 1
Conjugate | 2	Conjugate 2
Substrate	Substrate
Stop	Stop reagent

Bibliography

1. Corwin A. L. et al.: Shipboard impact of a probable Norwalk virus outbreak from coastal Japan. Am. J. Trop. Med. Hyg. 61(6), 898-903 (1999).
2. Daniels N. A. et al: A foodborne outbreak of gastroenteritis associated with Norwalk-like viruses: First molecular traceback to deli sandwiches contaminated during preparation. The Journal of Infectious Diseases, 181, 1467-70 (2000).
3. Gaulin C. et al.: Transmission of calicivirus by a foodhandler in the pre-symptomatic phase of illness. Epidemiol. Infect. 123, 475-478 (1999).
4. Jiang X. et al.: Outbreaks of gastroenteritis in elderly nursing homes and retirement facilities associated with human caliciviruses; Journal of Medical Virology 50: 335-341 (1996).
5. Jiang X. et al.: Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein; Journal of Virology Vol. 66 No. 11, 6527-6532 (1992).
6. Kohn M. A., MD, et al.: An outbreak of Norwalk virus gastroenteritis associated with eating raw oysters. Jama 273, 466-471 (1995).
7. Kukkula M. et al.: Outbreak of viral gastroenteritis due to drinking water contaminated by Norwalk-like viruses. The Journal of Infectious Diseases 180, 1771-1776 (1999).
8. Lodder W. J. et al.: Molecular detection of Norwalk-like caliciviruses in Sewage. Applied and Environmental Microbiology Vol. 65 No 12 , 5624-5627 (1999).
9. Otsu R. et al.: Detection of small round structured viruses in stool specimens from outbreaks of gastroenteritis by electron microscopy and reverse transcription-polymerase chain reaction. Acta virologica 44, 53-55 (2000).
10. Oyofo B. A. et al.: Norwalk-like virus and bacterial pathogens associated with cases of gastroenteritis onboard a U.S. navy ship. Am. J. Trop. Med. Hyg. 61(6), 904-908 (1999).
11. Payment P. et al.: Incidence of Norwalk virus infections during a prospective epidemiological study of drinking water related gastrointestinal illness. Can. J. Microbiol. 40, 805-809 (1994).
12. Pönkä A. et. al.: An outbreak of calicivirus associated with consumption of frozen raspberries. Epidem. Infect. 123; 469-474 (1999).
13. Prasad B. V. Venkataram et al.: X-ray crystallographic structure of the Norwalk virus capsid. Science Vol. 286, (1999).
14. Schreier E. et al.: Molecular epidemiology of outbreaks of gastroenteritis associated with small round structured viruses in Germany in 1997/98; Arch. Virol. 145, 443-453 (2000).
15. Taylor M. B. et al.: An epidemiological investigation of Norwalk virus infection in South Africa. Epidemiol. Infect. 116, 203-206 (1996).
16. Wright Peter J. et al.: Small round-structured (Norwalk-like) viruses and classical human caliciviruses in southeastern Australia, 1980-1996. Journal of Medical Virology 55, 312-320 (1998).
17. Zheng D.P. et al. : Norovirus classification and proposed strain nomenclature. Virology 346, 312-323 (2006).

18. Chan M.C.W. et al. : Fecal viral load and Norovirus associated gastroenteritis. Emerging infectious diseases (www.cdc.gov/eid) Vol. 12, No 8 (2006).
19. Román E. et al. : Acute viral gastroenteritis : proportion and clinical relevance of multiple infections in Spanish children. J.Med. Microbiol. 52, 435-440 (2003).
20. Okitsu-Negishi S. et al.: Detection of Norovirus Antigens from recombinant Virus-like particles and stool samples by a commercial Norovirus Enzyme-Linked Immunosorbent Assay Kit. J.Clin. Microbiol. 44, 3784-3786 (2006)
21. Castriciano S. et al. : Comparison of the RIDASCREEN® norovirus enzyme immunoassay to IDEIA NLV GI/GII by testing stools assayed by RT-PCR and electron microscopy. J.Virol.Methods (2007)