RIDASCREEN® Norovirus 3rd Generation,

Enzyme immunoassay (EIA) for the detection of Norovirus in human fecal samples

Catalog No. C1401US

R-Biopharm AG, An der neuen Bergstraße 17, 64297 Darmstadt, Germany
Tel: +49 (0) 61 51 - 81 02-0 / Fax: +49 (0) 61 51 - 81 02-20

INTENDED USE

The RIDASCREEN® Norovirus 3rd Generation EIA test is a qualitative enzyme immunoassay (EIA) intended for the detection of selected genogroup I (GI.1, GI.2, GI.3, GI.4, GI.7) and genogroup II (GII.1, GII.2, GII.3, GII.4, GII.5, GII.6, GII.7, GII.8, GII.10, GII.12, GII.13, GII.14, GII.17) norovirus strains in human feces as an aid in investigating the cause of acute gastroenteritis outbreaks. The likelihood of detecting a norovirus outbreak by use of the RIDASCREEN® Norovirus 3rd Generation EIA test improves as the number of patients tested during an outbreak increases, as well as when quality of the specimens increases. Preliminary identification of norovirus as the cause of an acute gastroenteritis outbreak by RIDASCREEN® Norovirus 3rd Generation EIA testing should be confirmed by reference methods as appropriate, particularly if only a limited number of positive samples are associated with a suspected outbreak. Additional testing of negative samples by other methods should be performed if norovirus is strongly suspected as the cause of an acute gastroenteritis outbreak.

SUMMARY AND EXPLANATION

Noroviruses are related, single stranded RNA, non-enveloped viruses in the genus *Norovirus*, family *Caliciviridae* that cause acute gastroenteritis in humans and other mammals. Noroviruses can be classified into five different genogroups of which genogroup I (GI) and genogroup II (GII) cause the majority of the infections in humans. In most years, GII viruses cause >75% of all norovirus outbreaks and typically >50% of these viruses belong to GII, cluster 4 (GII.4). Norovirus were previously described as “Norwalk-like viruses” (NLV).

Noroviruses are a major world-wide cause of gastroenteritis. They affect all ages, and are frequently involved in outbreaks in communal facilities, such as nursing homes, hospitals, day nurseries and prisons, and cruise ships[1, 2, 3].

Symptoms of norovirus infection are usually diarrhea, vomiting, stomach cramps, nausea, and fever. The disease is normally self-limiting and signs and symptoms may last for several days. In the young, elderly, and immunocompromised the disease may be life threatening due to dehydration. Common names associated with norovirus gastroenteritis are “winter vomiting disease”, stomach flu, acute non-bacterial gastroenteritis, and viral gastroenteritis.

Norovirus can only be cultured in very specialized cell culture systems[4]. Electron microscopy can be used to directly visualize norovirus in fecal specimens but is insensitive for detection relative to other methods of detection[5].

The RIDASCREEN® Norovirus 3rd Generation EIA test uses monoclonal antibodies against norovirus antigens to directly detect the presence of norovirus in fecal specimens. The RIDASCREEN® Norovirus 3rd Generation EIA test detects the most common causes of norovirus outbreaks in humans but does not detect all clinical strains, and may not be equally sensitive for all norovirus strains that can be detected.

Test sensitivity for detecting outbreaks is increased as the number of patients tested from an outbreak is increased; sensitivity is also increased if patient samples are collected as early as possible after symptom onset, preferably within 24 – 48 hours.

PRINCIPLE OF THE TEST

RIDASCREEN® Norovirus 3rd Generation test is a solid phase sandwich-type EIA for the detection of genogroups GI and GII noroviruses in stool samples. Microwell strips are coated with a mixture of GI and GII norovirus specific monoclonal antibodies. An aliquot of fecal suspension is added to the microwell together with biotinylated monoclonal norovirus antibodies. After washing, streptavidin peroxidase conjugate is added. Norovirus antigens that are present in the stool

sample are captured in sandwich complexes of the immobilized antibodies, the norovirus antigens and the monoclonal antibodies conjugated with biotin which are then recognized by the streptavidin peroxidase conjugates. Unbound streptavidin peroxidase conjugate is removed by washing and a chromogenic colorless substrate solution (hydrogen peroxide/TMB) is added. The substrate is hydrolyzed by any bound peroxidase, changing the chromogen to a blue color. Stopping the reaction with acid converts the blue to a yellow color indicating the presence of norovirus antigens.

Test results are read photometrically; intensity significantly above background levels is indicative of the presence of norovirus antigen in the specimen or control. The RIDASCREEN® Norovirus 3rd Generation EIA does not differentiate between norovirus strains.

MATERIALS SUPPLIED

Each RIDASCREEN® Norovirus 3rd Generation EIA kit contains sufficient materials for 96 direct specimens. The shelf life of the kit is as indicated on the outer box label.

1. 96 microwells (8 wells/strip; 12 strips) coated with norovirus monoclonal antibodies.
2. 1 vial (100 ml) Diluent 1, protein-buffered NaCl solution containing 0.1 % Kathon CG as preservative. **The Diluent 1 is also to be used as the negative control solution.**
3. 1 vial (100 ml) Wash buffer concentrate (10x), phosphate-buffered NaCl solution containing 0.1% thimerosal as preservative.
4. 1 vial (1.8 ml) Positive Control, noninfectious recombinant norovirus antigens.
5. 1 vial (10 ml) Conjugate 1, biotin-conjugated antibodies against noroviruses in stabilized protein solution.
6. 1 vial (10 ml) Conjugate 2, streptavidin peroxidase conjugate in stabilized protein solution.
7. 1 vial (10 ml) Substrate, solution containing hydrogen peroxide/TMB.
8. 1 vial (6 ml) Stop reagent, 1 N sulphuric acid. CAUTION: Avoid contact with skin. Flush with water if contact occurs.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

1. 1.5 to 2 ml reaction tubes, tube rack.
2. Deionized or distilled water.
3. Disposable pipettes.
4. Disposable inoculation loops.
5. Vortex mixer (optional).
6. Precision pipette for 50 -100 µl and 1000 µl.
7. Measuring cylinder (1000 ml).
8. Timer (minimum 1 hour).
9. Device for dispensing wash solution such as multi-channel pipette, syringe with manifold etc.
10. Microwell plate reader capable of reading absorbance at 450 nm.
11. Absorbent paper.

12. Disposable gloves.
13. Waste container containing 0.5 % hypochlorite solution.

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use only. This product is to be used by trained laboratory personnel only. Anyone performing an assay with this product must be experienced in performing similar laboratory procedures.

Safety Precautions:

1. Do not pipette samples or reagents by mouth. Avoid contact with broken skin or mucous membranes.
2. Do not smoke, eat or drink in areas where specimens or kit reagents are handled.
3. Wear disposable gloves while handling samples and wash hands after assay is complete.
4. Avoid skin contact with stop solution (1N sulphuric acid). It may cause irritation and burns. Flush with water if contact occurs.
5. The positive control in the kit contain noninfectious recombinant norovirus antigens. However, treat the control and the patient samples as potentially infectious. Patient specimen, positive control and all materials coming into contact with them should be handled at Biosafety Level 2 as recommended in the CDC/NIH manual “Biosafety in Microbiology and Biomedical Laboratories,” 2007.
6. Dispose of all materials used to perform the test by autoclaving at 121°C for at least 1 hour. Liquid waste may be disposed of by mixing with 0.5% hypochlorite solution for a minimum of 30 minutes. CAUTION: Liquid waste containing stop reagent must be neutralized before addition of hypochlorite solution.

Technical precautions:

1. **The instructions for carrying out the test must be strictly adhered to.** The detection of norovirus is dependent upon proper specimen collection, handling, transportation, storage and preparation, and failure to observe proper procedures during any of these steps can lead to incorrect results.
2. Kit reagents should be warmed to room temperature and gently mixed before use.
3. Avoid splashing or generation of aerosols.
4. Do not use RIDASCREEN® Norovirus 3rd Generation EIA reagents beyond the kit expiration date.
5. Each reagent has been optimized for maximum performance. Dilution or adulteration of these reagents may result in a loss of sensitivity. Incubation time and temperatures other that those specified may give erroneous results. Best results are obtained by adhering to the specifications.
6. Do not interchange or mix different lots of RIDASCREEN® Norovirus 3rd Generation EIA reagents.
7. Avoid microbial contamination of reagents or incorrect results may occur. Contamination of samples could cause erroneous results.

8. Use separate pipettes or pipette tips for each sample, control and reagent.
7. When dropping the reagent, the vial should be held vertically.
8. Do not allow the tips of any vial to touch the microwells in order to prevent cross-contamination.
9. Avoid scratching of coated wells. Rinse and fill all wells with care. While rinsing, check that all wells are filled evenly with Wash buffer, and that there are no residues in the wells.
10. Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as rinsing the test wells and thorough mixing of all prepared solutions
11. DO NOT REUSE MICROWELLS

SHELF LIFE AND STORAGE

The expiration date of the kit is given on the label. Do not use kit beyond the expiration date. Store kit reagents at 2-8 °C and promptly return to 2-8 °C after use. Return all unused microwells to their original pouch containing desiccant to exclude moisture before storage at 2-8 °C. Before use, keep microwells in pouch until the pouch reaches room temperature to avoid condensation. The substrate is light sensitive and should be protected from direct sunlight.

The following conditions may indicate reagent deterioration:

1. Any evidence of microbial contamination or heavy precipitation.
2. Any color in the substrate solutions before addition to microwells.
3. Damaged packaging of the microwell strips.
4. Leaking reagent vials.

If any of the above conditions are observed, contact the manufacturer or your local R-Biopharm distributor.

REAGENT PREPARATION

1. Bring all reagents, including the microwell pouch, to room temperature (20-25 °C) before use. Return all reagents to 2-8 °C immediately after use.
2. Prepare diluted Wash buffer (1x) by adding 1 part of Wash buffer concentrate (10x) to 9 parts of distilled or deionized water. Any crystals present in the concentrate must be dissolved beforehand in a water bath at 37 °C.
3. The microwells must be removed from the pouch shortly before use.
4. Prepare decontaminating vessel for discarding reagents and materials.
5. Do not allow microwells to dry between steps.
6. The test must not be carried out in direct sunlight.
7. Reproducibility of the assay is dependent on appropriateness with which the microwells are washed. Carefully follow the recommended washing sequence as outlined in the EIA test procedure.

SPECIMEN COLLECTION AND HANDLING

Stool specimens should be collected as soon as possible after onset of symptoms as peak viral counts are reported to occur on days 1-3 after onset of symptoms. Stool samples must be

collected in clean containers without any additives, as any of these may interfere with the RIDASCREEN® Norovirus 3rd Generation EIA test. Fecal specimens should be stored at 2-8 °C and tested within 72 hours. For long term storage of fecal specimens, -20 °C or colder is recommended. Before testing, frozen samples must be thoroughly thawed. Avoid repeating freeze-thawing. Do not store in self-defrosting freezers.

Samples diluted in Diluent 1, as per directions, may be stored at 2-8 °C for 3 days without affecting assay performance.

Samples should be tested fresh or frozen only once and thawed. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield incorrect results.

SPECIMEN PREPARATION

1. Add 1 ml of Diluent 1 into properly marked tube, using a disposable pipette or precision pipette.
2. Mix fecal sample thoroughly to ensure accurate representation of the specimen before pipetting:

   Liquid stool: Transfer 100 µl with a disposable pipette into the tube containing Diluent 1.

   Solid stool: Transfer approximately 100 mg (approx. 6 mm diameter, “pea size”) with a disposable inoculation loop into the tube containing Diluent 1.
3. Ensure proper homogenization by repeated pipetting of the suspension using a disposable pipette or, alternatively, by mixing on a vortex mixer avoiding aerosol generation.
4. Allow the homogenized stool suspension to settle for at least 10 minutes.
5. The supernatant of the stool suspension can be used directly in the test.

ASSAY PROCEDURE

1. After pouch has reached room temperature, remove the microplate strip holder including the microwell strips. Calculate the amount of strips needed for samples, positive control, and negative control (Diluent 1). Remove excessive strips and return to pouch. Reseal the pouch tightly and return to the refrigerator. If using retained strips place the appropriate amount of strips in the retained microplate strip holder.
2. Add 2 drops (100 µl) each of diluted fecal sample, positive control and negative control (Diluent 1) to the bottom of separate microwells.
3. Add 2 drops (100 µl) of Conjugate 1 to each microwell. Mix by gently swirling on tabletop.
4. Cover the microwells and incubate at room temperature (20 – 25 ºC) for 60 minutes.
5. Pour the liquid out of the wells into a discard vessel. Tap the plate upside down vigorously against absorbent paper to ensure complete removal of liquid from the microwells.
6. Add 300 µl of diluted Wash buffer (1x) to each microwell and pour the liquid out as in Step 5.
7. Repeat the washing procedure (Step 5 & 6) four more times (for a total of 5 washes). Do not allow the microwells to dry out at any time.
8. Add 2 drops (100 µl) of Conjugate 2 to each microwell. Mix by gently swirling on tabletop.

9. Cover the microwells and incubate at room temperature (20 - 25 ºC) for 30 minutes.
10. Repeat the washing procedure (Step 5, 6 & 7) for a total of 5 washes.
11. Add 2 drops (100 µl) of Substrate to each microwell. Mix by gently swirling on tabletop.
12. Cover the microwells and incubate at room temperature (20 - 25 ºC) for 15 minutes in the dark.
13. Add 1 drop (50 µl) of Stop reagent to each microwell. Mix by gently swirling on tabletop.
14. Spectrophotometrically read the absorbance of each microwell at 450 nm against an air blank within 10 minutes. Instructions for using appropriate photometers are to be observed; check adjustment of proper wavelength.

    NOTE: High positive fecal samples may cause the substrate to precipitate as black sediment. This will not affect the results.
15. If applicable: discard used microwells and retain the microplate strip holder for use with the remaining microwell strips.

QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. The user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable local, state, and federal regulations. Positive control and negative control (Diluent 1) are supplied with the kit. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. It is recommended that laboratories using the RIDASCREEN® Norovirus 3rd Generation EIA test participate in appropriate quality assessment trials.

The positive control and negative control (Diluent 1) must be used each time the test is performed to provide quality assurance of the procedure and the reagents. The negative control should be less than 0.200 at 450 nm and should be colorless. The positive control should be > 0.500 at 450 nm and should have a defined yellow color when read visually. For spectrometric determination, remove all bubbles and check the optical surface for spots or condensation; wipe with soft tissue if necessary. A value above 0.200 at 450 nm for the negative control may indicate insufficient washing. If the values for the controls differ from these specifications or if the Substrate is turbid or has turned blue before adding to the wells, the assay is considered invalid and should be repeated from the same sample preparation. If the stipulated values are not met, the following points must be checked before repeating the test:

- Expiration 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

If the specifications are not met after repeating the test, please consult the manufacturer or your local R-Biopharm distributor.

INTERPRETATION OF RESULTS

A positive control and negative control (Diluent 1) must be used each time the test is performed. The negative control should be less than 0.200 at 450 nm and should be colorless. The positive control should be > 0.500 at 450 nm and should have a defined yellow color. For spectrometric determination, remove all bubbles and check the optical surface for spots or condensation; wipe with soft tissue if necessary.

1. **Calculating the cut-off:** In order to establish the cut-off, 0.150 units are added to the measured absorbance for the negative control (Diluent 1).

   Cut-off = Absorbance for the negative control (Diluent 1) + 0.150.

2. **Test results:**
   a. Positive sample: Absorbance > than 10 % above the calculated cut-off.
   b. Negative samples: Absorbance > 10 % below the calculated cut-off.
   c. Indeterminate samples: Absorbance within ± 10 % of the cut-off.
      - For indeterminate results, a separate aliquot of the sample (or a fresh sample from the patient) should be retested and the second result reported. If the second result is indeterminate, the final result should be reported as indeterminate.

Examples:

In an assay run the cut-off is determined with 0.200 units (absorbance for the negative control (0.050) + 0.150).

Measured sample absorbance	Cut-off	Cut-off range (cut-off ±10 %)	Interpretation
1.200	0.200	0.180 - 0.220	1.200 > 0.220 positive sample
0.050	0.200	0.180 - 0.220	0.050 < 0.180 negative sample
0.190	0.200	0.180 - 0.220	0.180 < 0.190 < 0.200 indeterminate sample (repeat as above)

LIMITATIONS

1. Performance of the RIDASCREEN® Norovirus 3rd Generation EIA test for detecting norovirus infection in individual patients is limited and a negative result should not exclude the possibility of norovirus infection for an individual patient during an outbreak. Results should also be carefully interpreted as outbreaks may also be incorrectly identified as present due to falsely positive results (see *Clinical Performance: Clinical Evaluation*, below). In addition to the inherent limitations of the test, false-negative results may also occur due to collection of specimens at a time in the disease course when too few virions are present for detection or because of improper handling of specimens. Results of testing with the RIDASCREEN® Norovirus 3rd Generation EIA test should be confirmed by testing with reference assays.

   Interpretation of results obtained with the RIDASCREEN® Norovirus 3rd Generation EIA test must be used in conjunction with the clinical symptoms, medical history and other clinical and/or laboratory findings of the patients being tested to appropriately identify a norovirus outbreak.

2. The RIDASCREEN® Norovirus 3rd Generation EIA test detects genogroup specific viral proteins present in human genotypes of genogroup I and genogroup II norovirus. The test cannot be used to differentiate between norovirus strains. Test sensitivity may differ for different genogroups and for specific subtypes within each genogroup.

3. The RIDASCREEN® Norovirus 3rd Generation EIA test detects norovirus antigens of the human pathogenic genogroups GI and GII in stool specimens. In two studies the following norovirus genotypes were detected using the RIDASCREEN® Norovirus 3rd Generation EIA test: GI.1, GI.2, GI.3, GI.4, GI.7, GII.1, GII.2, GII.3, GII.4, GII.5, GII.6, GII.7, GII.8, GII.10, GII.12, GII.13, GII.14, GII.17. As the assay has not been validated with all known GI and GII genotypes, a failure to detect norovirus may be due to antigenic diversity.

4. Monoclonal antibodies may fail to detect strains of norovirus which have undergone amino acid changes in the target epitope region. Over time, antigenic changes in circulating norovirus strains, or the emergence of new norovirus strains, may affect performance of the RIDASCREEN® Norovirus 3rd Generation EIA test.

5. A positive test result for the presence of norovirus does not exclude the presence of other infectious pathogens; one or more pathogens may be present in an individual patient and contribute to clinical symptoms. Additional microbiological tests should be performed in parallel with the RIDASCREEN® Norovirus 3rd Generation EIA test in order to exclude other possible causes of illness as appropriate.

6. The detection of norovirus is dependent upon proper specimen collection, handling, transportation, storage and preparation. Failure to observe proper procedures during any of these steps can lead to incorrect test results, most likely a falsely negative result.

EXPECTED VALUES

Noroviruses are a frequent cause of gastroenteritis outbreaks in semi-closed settings that involve children and/or adults such as day-care centers, nurseries, hospitals, nursing homes, prisons, and cruise ships, or other settings that may facilitate person-person spread(6). Noroviruses account for ~50% of all acute gastroenteritis outbreaks worldwide, although the proportion of outbreaks expected to be positive and the rate of positive test results within an outbreak depend on a number of factors. These factors may include (among others), the prevalence of norovirus within the population, the specific genotype(s) circulating, how rapidly specimens are taken, and the setting of the outbreak. Sample collection, transport, and storage may also affect the rate of positive test results. Specimens should be obtained from symptomatic patients as soon as possible after an outbreak is suspected to maximize the likelihood of identifying a true outbreak.

PERFORMANCE CHARACTERISTICS

Clinical evaluation

Performance of the RIDASCREEN® Norovirus 3rd Generation EIA test was compared to a reference standard of conventional RT-PCR testing of stool specimens.[1] Specifically, specimens were tested by conventional RT-PCR followed by bi-directional sequencing for both Region B and Region D norovirus sequences. In addition a subset of patient samples was also tested by electron microscopy. A total of 609 fecal samples from patients with suspected infection were included in the evaluation of diagnostic accuracy. The overall performance of the RIDASCREEN® Norovirus 3rd Generation test is shown in Table 1.

[1] One patient who had Norovirus detected by EM but was negative by PCR methods was analyzed as reference positive in Table 1 below.

Table 1. Clinical Performance of the RIDASCREEN® Norovirus 3rd Generation EIA Test[2]

	RT-PCR-Sequencing and EM Positive Reference Standard		
RIDASCREEN® Norovirus 3rd Generation EIA Test	**Positive**	**Negative**	**Total**
Positive	159	37	196
Negative	85	328	413
Total	244	365	609

Positive agreement (95% CI)	159/244	65% (59-71%)
Negative agreement (95% CI)	328/365	90% (86-93%)

Of the 244 reference standard positive samples, 167 samples (114 RIDASCREEN positive) underwent genogroup and subtype identification. Results were as follows:

Table 2. Clinical Trial Genogroup and Subtype Identification

Genogroup I			**Genogroup II**		
Subtype	No. of PCR positive samples	No. of samples detected by ELISA	Subtype	No. of PCR positive samples	No. of samples detected by ELISA
2	2	1	1	1	1
3	10	2	3	3	3
3b	2	0	4	123	96
4	8	6	7	1	1
5	1	0	Unable to type	5	4
Unable to type	11	0			
Total	**34**	**9**	**Total**	**133**	**105**

Sensitivity of ELISA-based tests for detecting norovirus outbreaks increases as the number of patients tested during a suspect outbreak increases; the Centers for Disease Control and prevention (CDC) has published recommendations regarding the number and quantity of stool specimens that should be tested during suspected norovirus outbreaks, as well as recommendations regarding timing of specimen collection.(5) Local public health authorities and/or CDC should be consulted if the cause of an acute gastroenteritis outbreak is uncertain.[3] Preliminary identification of norovirus as the cause of an acute gastroenteritis outbreak by RIDASCREEN® Norovirus 3rd Generation EIA testing should be confirmed by reference methods as appropriate, particularly if only a limited number of positive samples are associated with a suspected outbreak. Additional testing of negative samples by other methods should be performed if norovirus is strongly suspected as the cause of an acute gastroenteritis, particularly if a limited number of samples are available during a suspected outbreak or if samples were collected more than 72 hours after the onset of symptoms.

[2] Study samples were collected from patients during outbreaks and as sporadic cases; overall performance is combined for both groups.
[3] Confirmed food or waterborne norovirus outbreaks are nationally reportable diseases.

Analytical sensitivity

The limit of detection of the RIDASCREEN® Norovirus 3rd Generation EIA test was determined with 2 native fecal samples (GI.1 and GII.4). A 20% w/v suspension and 6 ten-fold serial dilutions were prepared and tested in triplicate by EM, EIA, rRT-PCR, RT-PCR/ RegionB and RT-PCR/ Region D. The limit of detection of the EIA was established as the lowest number of viral particles (based on EM) and norovirus RNA copies/g (based on rRT-PCR) that tested positive in at least 2 of 3 replicates.

Table 3. Minimum Number of Detectable Virus Particles and NoV RNA Copies Required for a Positive Signal in the EIA (limit of detection)

Sample	NoV Concentration in the Original (Undiluted) Specimen		Minimum Detectable NoV Concentration by EIA[a]		RT-PCR[b]	
	NoV Particle Numbers per Gram of Fecal Sample[c]	NoV RNA Copies per Gram of Fecal Sample[d]	NoV Particle Numbers per Gram of Fecal Sample	NoV RNA Copies per Gram of Fecal Sample	Region B	Region D
A (GI.1)	5.0E+09	3.1E+09	2.4E+07	5.45E+06	positive	positive
B (GII.4)	4.4E+09	1.6E+10	1.6E+07	2.89E+07	positive	positive

[a] Number of NoV particles or NoV RNA copies per gram of fecal sample in the highest dilution positive by EIA.
[b] RT-PCR/Region B or RT-PCR/Region D result in the highest dilution positive by EIA.
[c] NoV particle numbers determined by EM.
[d] NoV RNA copy numbers determined by rRT-PCR.

Interfering substances

The following substances had no effect on the test results when they were mixed with fecal samples with an analyte concentration near the assay cutoff, and one negative fecal sample at the specified concentrations:

Table 4. Interfering Substances

Substance	**Concentration**
Mucin	5 % (w/w)
Human blood	5 % (v/w)
Barium sulfate (contrast medium)	5 % (w/w)
Loperamide (anti-diarrhea drug)	5 % (w/w)
Pepto-Bismol (anti-diarrhea drug)	5 % (v/w)
Stearic acid / Palmitic acid (1:1) (fatty acids)	40 % (w/w)
Metronidazole (0.5 % solution) (antibiotic)	5 % (v/w)
Diclofenac (analgesic)	0.00263 % (v/w)
Cyclamate (artificial sweetener)	5 % (v/w)

Cross-reactivity

The cross-reactivity of the RIDASCREEN® Norovirus 3rd Generation EIA test was evaluated by examining the reactivity of a wide range of common intestinal bacteria, parasites, and viruses in the assay. Native specimens from patients suffering from infections with the respective pathogen, undiluted bacterial cultures (ranging from 10^6 to 10^9 organisms per ml) or cell culture supernatants were used to analyze the cross-reactivity. None of organisms tested in the table below produced a positive result.

Table 5. Organisms that Do Not React with RIDASCREEN® Norovirus 3rd Generation EIA Test

Microrganism (Number tested)	Microrganism (Number tested)
Acinetobacter lwoffi (1)	*Morganella morganii* (1)
Adenovirus (2)	*Proteus mirabilis* (2)
Aeromonas hydrophila anaerogenes (1)	*Proteus vulgaris* (1)
Aeromonas hydrophila hydrophila (1)	*Providencia stuartii* (1)
Astrovirus (2)	*Pseudomonas aeroginosa* (1)
Campylobacter coli (1)	*Pseudomonas fluorescens* (2)
Campylobacter fetus (1)	*Pseudomonas putida* (1)
Campylobacter jejuni (1)	Rotavirus (2)
Candida albicans (1)	*Salmonella Agona* (1)
Citrobacter sp. (1)	*Samonella choleraesuis* (1)
Citrobacter freundii (1)	*Salmonella enteritidis* (2)
Clostridium difficile (1)	*Salmonella infantis* (1)
Clostridium sordellii (1)	*Salmonella Ohio* (1)
Cryptosporidium parvum (1)	*Salmonella typhimurium* (1)
Ent. Histolytica (1)	Sapovirus (1)
Enterobacter cloacae (1)	*Serratia proteamaculans (liquefaciens)* (1)
Enterococcus faecalis (1)	Shigatoxin STX1 (1)
Enterococcus faecium (1)	Shigatoxin STX2 (1)
Giardia lamblia (1)	*Shigella flexneri* (1)
E. coli (8)	*Shigella sonnei* (1)
E. hermannii (1)	*Staphylococus aureus* (1)
Helicobacter pylori (2)	*Streptococcus agalactiae* (1)
Lactococcus lactis (1)	*Streptococcus dysgalactiae* (1)
Listeria innocua (1)	*Streptococcus uberis* (1)

Reproducibility and precision

Reproducibility and precision of the RIDASCREEN® Norovirus 3rd Generation EIA test were determined using six reference samples covering the whole assay range from negative to high positive. The Intra-assay reproducibility was determined by measuring 40 replicates of each reference. Mean values and coefficient of variance (CV) were determined for three independent kit lots. For the Inter-assay precision the references were run in triplicates on 10 consecutive days. The measurements were conducted using three different independent kit lots and were performed by three different operators. The Inter-lot precision was calculated over three kit lots. The 90 measurements (triplicates, 10 days, three kit lots) for each sample from negative to high positive were evaluated by analysis of variance (ANOVA 2-factorial). The experiments were done by three different technicians.

Table 6. Intra-Assay Reproducibility and Inter-Assay/Lot Precision

References Mean / CV	Intra-Assay Reproducibility			Inter-Assay Precision			Inter-Lot Precision
	Kit Lot 1	Kit Lot 2	Kit Lot 3	Kit Lot 1	Kit Lot 2	Kit Lot 3	Kit Lot 1-3
negative	0.051 / 4.35%	0.051 / 4.41%	0.051 / 5.11%	0.085 / 28.02%	0.092 / 20.45%	0.084 / 20.86%	0.087 / 23.26%
low positive #1	0.563 / 3.80%	0.561 / 3.72%	0.559 / 3.78%	0.616 / 10.11%	0.705 / 16.93%	0.666 / 12.08%	0.662 / 14.84%
low positive #2	0.309 / 5.16%	0.308 / 5.14%	0.307 / 5.19%	0.460 / 12.54%	0.506 / 10.04%	0.492 / 15.66%	0.486 / 13.33%
medium positive #1	1.205 / 9.48%	1.198 / 9.50%	0.953 / 6.92%	1.108 / 7.55%	1.181 / 5.89%	1.213 / 9.28%	1.167 / 8.82%
medium positive #2	1.014 / 3.39%	1.012 / 3.42%	1.006 / 3.43%	0.882 / 16.09%	0.920 / 9.53%	0.924 / 17.10%	0.909 / 14.60%
high positive	1.653 / 2.54%	1.643 / 2.50%	1.481 / 6.81%	1.533 / 11.66%	1.690 / 7.33%	1.716 / 10.53%	1.646 / 11.23%

Inter-site precision

The Inter-site precision was evaluated at three testing sites using six references covering the whole assay range from negative to high positive. The references were measured in triplicates at 5 days with two runs per day. Precision was evaluated by analysis of variance.

Table 7. Inter-Site Precision

Reference	High Positive	Medium Positive	Low Positive #1	Low Positive #2	Low Positive #3	Negative
	Mean / CV	Mean / CV	Mean / CV	Mean / CV	Mean / CV	Mean / CV
Site 1 -3	1.372 / 16.01%	1.054 / 20.13%	0.687 / 20.07%	0.615 / 17.67%	0.373 / 23.30%	0.080 / 40.34%

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Revision 04-Sep-2018

Manufactured by

R-Biopharm AG
An der neuen Bergstraße 17, 64297 Darmstadt
Tel.: +49 - 61 51 - 8102 0 / Fax: +49 - 61 51 - 8102 20
E-Mail: info@r-biopharm.de / www.r-biopharm.com

Authorized Representative USA

R-Biopharm Inc.
870 Vossbrink Dr.
Washington, MO 63090
Phone: (877) 789 - 3033
Fax: (866) 922 - 5856
E-mail: info@r-biopharm.com