RIDASCREEN® Rotavirus

REF C0901

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1. Intended use

For *in vitro* diagnostic use. RIDASCREEN® Rotavirus is an enzyme immunoassay for qualitative identification of rotaviruses in human stool samples.

2. Summary and explanation of the test

Rotaviruses are the major pathogens involved in non-bacterial gastroenteritis among children in the age group 6 months to 3 years. They may also be identified as the cause of disease in older children and adults. In the risk groups – children, elderly, and immunosuppressed patients – these infections can be fatal.
Rotavirus infections occur mainly in the winter months. Endemics and epidemics with several thousand patients have been described as well. In hospitalized children with acute enteritis, up to 50 % of the examined samples are found to be rotavirus positive. Rotaviruses are transferred by the fecal-oral route and they are excreted by the intestines in large amounts, so nosocomial rotavirus infections are feared greatly in neonatal care units and pediatric clinics, and they are difficult to manage. This means that an early and reliable method of identification of the rotavirus is very important, in order to prevent further infection.
It was not possible to detect rotaviruses until stool samples and biopsy material from the intestines could be examined by electron microscopy – today this is the standard method of identification. Another development is the identification in cell cultures *in vitro*. Because that is difficult and time-consuming, however, it is not used routinely to identify the rotavirus. Serological tests only help to confirm a diagnosis of rotavirus enteritis, because the IgM antibodies are not detectable before Day 5 after disease begin. An early diagnosis is ruled out with that method.
Der RIDASCREEN® Rotavirus Test is a simple and highly sensitive enzyme immunoassay method which makes early and reliable identification of rotavirus antigens possible. Larger sample sizes can also be processed in shorter periods of time.

3. Test priniple

The RIDASCREEN® Rotavirus Test employs monoclonal antibodies in a sandwich-type method. A monoclonal antibody to the product of the 6th viral gene (VP6) is coated to the well surface of the microwell plate. This is a group specific antigen that is found in all *rotaviruses* that cause disease in humans. A pipette is used to place a suspension of the stool sample to be examined as well as control specimens into the well of the microwell plate together with biotinylated monoclonal anti-rotavirus antibodies (Conjugate 1) for incubation at room temperature (20 - 25 °C). 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 rotaviruses in the stool sample, a sandwich complex will form which consists of immobilized antibodies, the rotavirus 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 rotaviruses 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-rotavirus antibodies (mouse)
Diluent | 1	100 ml	Sample dilution buffer, protein-buffered NaCl solution; ready to use, blue colored
Wash buffer	100 ml	Wash buffer, phosphate buffered NaCl solution (concentrated 10-fold); contains 0.1 % thimerosal
Control | +	2 ml	Positive control; inactivated rotavirus culture; ready for use
Control | -	2 ml	Negative control (sample dilution buffer), ready for use
Conjugate | 1	13 ml	Biotin-conjugated monoclonal antibodies (mouse) to the rotaviruses in stabilized protein solution; ready to use, red 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

Dangerous substances are indicated according to labelling obligations. For more details, refer to Safety Data Sheets (SDS) at www.r-biopharm.com.

5. Storage instructions

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 aluminum 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 aluminum bag and immediately stored at 2 - 8 °C.
The colorless Substrate must also 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. Reagents required but not provided

6.1 Necessary reagents

The following reagents are required to perform the RIDASCREEN® Rotavirus test:

Reagents
Distilled or deionized water

6.2 Necessary laboratory equipment

The following equipment is required to perform the RIDASCREEN® Rotavirus test:

Equipment
Test tubes
Disposable pipettes (Article no.: Z0001)
Vortex mixer (optional, see 9.3.)
Micropipette for 50 - 100 µl and 1 ml volume
Measuring cylinder (1,000 ml)
Stop clock
Washing device for microtiter plates or multichannel pipette (300 µl).
Photometer for microtiter plates (450 nm, reference filter 620-650 nm)
Filter paper (laboratory towels)

7. Warnings and precautions

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 Safety Data Sheets (SDS) at www.r-biopharm.com.

The kit includes a positive control that contains an inactivated rotavirus culture. It must be treated as potentially infectious material and handled in accordance with the national safety regulations, just like the patient samples.

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. Collection and storage of specimens

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 2 - 8 °C for use within three days (Tab. 1).

Tab. 1: Specimen storage

Undiluted stool specimen		Diluted stool specimen
2 - 8 °C	≤ - 20 °C	2 - 8 °C
≤ 3 days	> 3 days	≤ 3 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® Rotavirus 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 testing of stool samples from contact persons who do not exhibit clinical symptoms, in order to identify asymptomatic carriers.

9. Test procedure

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 aluminum 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 washing buffer

Mix 1 part wash buffer concentrate Wash buffer 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 specimens

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 that case, it is advisable to centrifuge the sample at 2,500 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 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 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).

Note: High-positive patient samples may cause black-colored precipitates of the substrate.

10. Quality control – indication of instability or deterioration of reagents

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 (OD) 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 coloration 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. Evaluation 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. Teste 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® Rotavirus Test identifies antigens of the rotaviruses in stool samples. 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 signs and symptoms.

A positive result does not rule out the presence of other infectious pathogens.

A negative result does not rule out the possibility of *rotavirus* infection. Such a result may be due to intermittent excretion of the virus, or the amount of antigen in the sample may be too small. If the patient history supports a suspicion of *rotavirus* infection, the examination should be repeated with another stool sample.

A borderline result may be due to non-homogeneous distribution of viruses in the stool sample. In this case, examination should either be repeated with a second suspension from the same sample or another stool sample should be requested.

13. Test performance

13.1 Test quality

The RIDASCREEN® Rotavirus was validated by comparison with three commercial rotavirus ELISAs. The sample collective that was used consisted of fresh, same-day samples taken at a routine laboratory and of prepared samples that had been frozen in advance at -20 °C for use in the comparison study. One homogeneous baseline suspension was tested by each of the ELISAs in accordance with the manufacturers' instructions. A sample was considered positive or negative, if the results of two out of three reference tests were in agreement. The results of that study are summarized in Table 2.

Tab. 2: Correlation between RIDASCREEN® Rotavirus ELISA and three other commercial ELISAs

	Competitor-ELISA		
RIDASCREEN® Rotavirus	+	-	Total
+	22	1*	23
-	1*	112	113
Total	23	113	136

* Both samples were negative in the Rotavirus PCR Test.

Sensitivity : 95,7 % Specificity : 99,1 %

13.2 Cross-reactivity

A variety of pathogenic microorganisms from the intestinal tract were examined with the RIDASCREEN® Rotavirus ELISA and showed no cross reactivity.
These studies were conducted with bacterial suspensions shown to have concentrations of 10^6 to 10^9 organisms per ml. Virus culture supernatants and toxins as well as stool samples are listed accordingly. The results of that study are summarized in Table 3.

Tab. 3: Cross reactivity with pathogenic microorganisms

Organism	Origin	Source	[OD 450 nm] mean value
Acinetobacter lwoffii	Culture	DSM 2403	0.078
Aeromonas hydrophila anaerogenes	Culture	DSM 30020	0.068
Aeromonas hydrophila hydrophila	Culture	DSM 30016	0.078
Citrobacter sp.	Culture	DSM 30047	0.055
Citrobacter freundii	Culture	DSM 30039	0.090
Enterobacter cloacae	Culture	DSM 30054	0.072
Enterococcus faecalis	Culture	DSM 2570	0.066
Enterococcus faecium	Culture	DSM 20477	0.095
E. coli	Culture	LMU Munich	0.069
E. coli	Culture	LMU Munich	0.082
E. coli	Culture	LMU Munich	0.083
E. hermannii	Culture	DSM 4560	0.054
Lactococcus lactis	Culture	DSM 20481	0.073
Listeria innocua	Culture	DSM 20649	0.078
Proteus mirabilis	Culture	DSM 788	0.053
Proteus mirabilis	Culture	DSM 4479	0.065
Proteus vulgaris	Culture	DSM 30119	0.053
Providencia stuartii	Culture	DSM 6676	0.080
Pseudomonas aeruginosa	Culture	DSM 939	0.065
Pseudomonas fluorescens	Culture	DSM 4358	0.061
Pseudomonas fluorescens	Culture	DSM 50124	0.082
Pseudomonas putida	Culture	DSM 291	0.058
Salmonella agona	Culture	LMU Munich	0.097
Salmonella choleraesuis	Culture	DSM 4224	0.088
Salmonella infantis	Culture	LMU Munich	0.059
Salmonella ohio	Culture	LMU Munich	0.061
Salmonella typhimurium	Culture	DSM 554	0.052
Serratia liquefaciens	Culture	DSM 4487	0.050
Shigella flexneri	Culture	DSM 4782	0.063
Shigella sonnei	Culture	DSM 5570	0.048
Staphylococcus aureus	Culture	DSM 20372	0.063
Streptococcus agalactiae	Culture	DSM 2134	0.078
Streptococcus dysgalactiae	Culture	DSM 20662	0.079

Streptococcus uberis	Culture	DSM 20569	0.072
E. coli (O157:H-)	Culture	LMU Munich	0.097
E. coli (O116:H21)	Culture	LMU Munich	0.074
E. coli (O111:H-)	Culture	LMU Munich	0.084
E. coli (O22:H8)	Culture	LMU Munich	0.095
E. coli (O26:H11)	Culture	LMU Munich	0.086
Candida albicans	Culture	ATCC 10231	0.082
Salmonella enteritidis	Culture	DSM 9898	0.065
Campylobacter jejuni	Culture	DSM 4688	0.054
Campylobacter coli	Culture	DSM 4689	0.073
Campylobacter fetus	Culture	DSM 5361	0.064
Helicobacter pylori	Culture	DSM 4867	0.053
Morganella morganii	Culture	DSM 6675	0.053
Astrovirus	Culture supernatant	Micromun	0.055
Astrovirus	Stool	TU Dresden	0.080
Adenovirus	Culture supernatant	Micromun	0.065
Adenovirus	Stool	TU Dresden	0.061
H. pylori Probe	Inactivated *H. pylori* lysate	Kit control RIDASCREEN® H. pylori FemtoLab	0.082
C. perfringens 50 µg/ml	Toxoid	Kit control C. perfringens Enterotoxin A	0.058
Shigatoxin STX1	Toxoid	Toxin Technology	0.064
Shigatoxin STX2	Toxoid	Toxin Technology	0.073
C. sordellii	Culture	tgcBiomics	0.060
C. difficile	Culture	VPI 1640	0.060
Cryptosporidium parvum	Culture	Waterborne Inc.	0.052
Campylobacter	Stool	Routine lab	0.042
Giardia lamblia	Stool	TI Berlin	0.037
Entamoeba histolytica	Stool	TI Berlin	0.049
Salmonella enteritidis	Stool	Routine lab	0.066
Sapovirus	Stool	TU Dresden	0.062

13.3 Precision

The reproducibility of the RIDASCREEN® Rotavirus ELISA was tested with six references representing the complete measurement range from weak to high positive. To determine the intra-assay reproducibility, 40 replicates of these references were assayed. Kit 1 was assayed with reference standard 1 (RS 1), and Kits 2 and 3 were assayed with reference standard 2 (RS 2). The mean values and the variation coefficients (VC) were determined for all three lots.
For the inter-assay reproducibility, references from ten different working days were assayed in duplicates, with two runs per day. The measurements were determined in three lots by three technicians.
The inter-lot reproducibility was determined for two lots (Lots 2 and 3). The results of that study are shown in Table 4.

Tab. 4: Reproducibility and precision of the RIDASCREEN® Rotavirus ELISA

Reference Mean value / CV(%)		**Intra-assay**			**Inter-assay**			**Inter-lot**
		Kit Lot 1 (RC 1)	Kit Lot 2 (RC 2)	Kit Lot 3 (RC 2)	Kit Lot 1 (RC 1)	Kit Lot 2 (RC 2)	Kit Lot 3 (RC 2)	Kit Lot 2-3
1	MV	2.279	2.116	2.300	2.206	1.902	2.035	1.968
	CV (%)	4.82 %	9.38 %	6.86 %	11.78 %	20.30 %	18.33 %	19.42 %
2	MV	1.318	1.311	1.535	1.642	1.273	1.389	1.331
	CV (%)	8.87 %	10.67 %	5.00 %	14.82 %	25.42 %	21.61 %	23.70 %
3	MV	1.265	1.184	1.517	1.483	1.117	1.219	1.168
	CV (%)	9.82 %	13.36 %	5.25 %	15.00 %	22.68 %	21.43 %	22.01 %
4	MV	0.837	0.660	0.853	1.116	0.710	0.781	0.745
	CV (%)	10.26 %	15.12 %	4.53 %	17.26 %	25.81 %	24.37 %	25.36 %
5	MV	0.658	0.544	0.670	0.738	0.548	0.607	0.578
	CV (%)	9.94 %	14.99 %	4.85 %	17.78 %	25.43 %	26.14 %	26.21 %
6	MV	0.373	0.298	0.509	0.532	0.386	0.435	0.411
	CV (%)	9.85 %	15.38 %	7.43 %	17.94 %	28.90 %	27.73 %	28.87 %

13.4 Analytical Sensitivity

The detection limit of RIDASCREEN® Rotavirus ELISA was determined with the serial dilution of a stool sample quantified by immunoelectron microscopy (IEM). The measurements were taken in triplicate, based on a virus titer of 10.6×10^7 particles/ml. The detection limit was defined as 6.63×10^3 virus particles/ml of the

stool sample. Results of the titration series are shown in Table 5. Note that the positive OD value in ELISA is caused by intact virus particles, but also by fragments of the virus, which are not counted in the IEM.

Tab.5: Determination of the analytical sensitivity of RIDASCREEN® Rotavirus ELISA

IEM	RIDASCREEN® Rotavirus	
Virus particles / ml	Mean value [OD 450]	Results
5.3 x 10^6	4.036	positive
5.3 x 10^5	4.043	positive
2.65 x 10^5	4,051	positive
1.325 x 10^5	4.060	positive
0.663 x 10^5	3.140	positive
5.3 x 10^4	2.022	positive
2.65 x 10^4	0.788	positive
1.325 x 10^4	0.451	positive
0.663 x 10^4	0.240	positive
0.332 x 10^4	0.124	negative
0.165 x 10^4	0.049	negative

13.5 Interfering substances

The following list of substances showed no effects on the test results when they were blended into *rotavirus* positive and *rotavirus* negative stool samples in the described concentrations:

Mucins	5.0 % w/w	Diclofenac	0.00263 % v/w
Human blood	5.0 % v/w	Cyclamate	5.0 % v/w
Barium sulfate	5.0 % w/w	Stearic acid and palmitic acid combination	40 % w/w (1:1)
Loperamide	5.0 % w/w		
Pepto-Bismol	5.0 % v/w	Metronidazole 0.5 % solution	5.0 % v/w

14. Version history

Version number	Chapter and designation
2017-04-20	Previous version
2019-07-08	General revision 4. Reagents provided 8. Collection and storage of specimens 9.2 Preparing the washing buffer 9.5 Washing

15. Explanation of symbols

General symbols

IVD	For in vitro diagnostic use
	Consult instructions for use
LOT	Lot number
	Expiry
	Store at
REF	Article number
Σ	Number of tests
	Date of manufacture
	Manufacturer

Test specific symbols

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

16. References

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