RIDASCREEN® Astrovirus

Article no.: C1301

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1. Intended use

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

2. Summary and explanation of the test

Astroviridae were first described by Appleton and Higgins in 1975. Using an electron microscope, Madeley and Cosgrove then made this small, round virus visible in stool samples from children who had diarrhea, and they named it for the star shape of its appearance. Together with *Caliciviridae* and several other viruses, it is one of the so-called Small Round Structured Viruses (SRSV) which show remarkably structured surfaces, opposed to the group known as Small Round Viruses (SRV), which present smooth, unstructured surfaces. Among others, SRV also include the *Parvovirus* and *Picornavirus* families.

The epidemiological significance of gastroenteritis caused by the *Astrovirus* was very poorly understood during the years when use of the electron microscope was the only way to detect the virus, and the frequency of astrovirus infections with diarrhea or diarrhea combined with vomiting was greatly underestimated. This was partly due to the relatively low sensitivity of the electron microscope (EM), but also because it was not always possible to clearly differentiate the SRSV particles. The low incidence rate of 1% did not match the available sero-epidemiological data, which indicated that up to 70% of older children and young adults carried specific anti-astrovirus antibodies.

Newer methods such as enzyme immunoassay, which is 10 to 100 times more sensitive than EM, and the highly sensitive polymer chain reaction (PCR) method with detection limits down to 10^2 particles per stool sample, have increasingly raised the significance and participation of the astrovirus in the differential diagnosis of diarrhea cases. Today the astrovirus incidence in cases of acute diarrhea ranges from 2.5% to 10%, so it is in the frequency range known for adenovirus infections (Type 40/41). After norovirus and rotavirus infections, the astrovirus is the third most frequent cause of non-bacterial gastroenteritis.

Of the 8 serotypes known today, serotypes 1 to 5 are particularly relevant. Gastroenteritis caused by the astrovirus may occur in all age groups, though it is most frequently seen in children and older persons. Outbreaks of this infection occur most often in kindergartens, schools, hospitals, and homes for the elderly, but gastroenteritis due to the astrovirus also occurs spontaneously in the military environment and in travel groups. It is as infectious as the rotavirus. The infection is transmitted via contaminated food (oysters in particular), through the water supply, and by the fecal-oral transmission route.

RIDASCREEN® Astrovirus ELISA employs highly specific antibodies to ensure reliable identification of astrovirus antigens stool samples.

3. Test principle

The RIDASCREEN® Astrovirus Test uses specific antibodies in a sandwich-type method. Specific antibodies to all known astrovirus serotypes are coated to the well surface of the microwell plate. 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 specific anti-astrovirus antibodies (Conjugate 1) for incubation at room temperature (20-25 °C). After a wash step, streptavidin poly-peroxidase conjugate (Conjugate 2) is added and that is incubated again at room temperature (20–25 °C). With the presence of astroviruses in a stool sample, a sandwich complex will form which consists of immobilized antibodies, the astrovirus 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 astroviruses found in the specimen.

4. Reagents provided

The reagents in the kit are sufficient for 96 determinations.

Plate	96 det.	Microwell plate, 12 microwell strips (which can be divided) in the strip holder; coated with specific anti-astrovirus antibodies
Diluent | 1	100 ml	Sample dilution buffer, protein-buffered NaCl solution, ready to use, blue colored
Wash	100 ml	Wash buffer, phosphate buffered NaCl solution (concentrated 10-fold); contains 0.1% thimerosal
Control | +	2 ml	Positive control, inactivated astrovirus culture; ready for use
Control | -	2 ml	Negative control (sample dilution buffer); ready for use
Conjugate | 1	13 ml	Biotin-conjugated specific anti-astrovirus antibodies in stabilized protein solution; ready for use; yellow 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 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 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. 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 (1,000 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 0.5% hypochlorite solution

7. Precaution for users

For *in vitro* diagnostic use 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 kit includes a positive control that contains an inactivated astrovirus culture. 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

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.
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® Astrovirus 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 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. In the case of solid stool samples, add an equivalent amount of the stool sample (approx. 50–100 mg) with a spatula or disposable inoculation loop and suspend.
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 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 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, what means 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 (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. 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® Astrovirus Test identifies antigens of the astrovirus 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 picture.

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

A negative result does not rule out the possibility of astrovirus 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 astrovirus infection, the examination should be repeated with another stool sample.

A marginal 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. Performance characteristics

13.1. Test quality

A retrospective validation study with the RIDASCREEN® Astrovirus ELISA examined 92 stool samples. The samples were homogenized and underwent comparative examination by RIDASCREEN® Astrovirus ELISA and another commercial ELISA. The results of that examination are summarized in Table 1.

Table 1: Correlation of RIDASCREEN® Astrovirus ELISA to another commercial ELISA

		ELISA	
		pos	**neg**
RIDASCREEN® Astrovirus	**pos**	31	0
	neg	0	61

Positive agreement: 100 %
Negative agreement: 100 %

13.2. Cross reactivity

A variety of pathogenic microorganisms from the intestinal tract were examined with the RIDASCREEN® Astrovirus ELISA and showed no cross reactivity. These studies were

conducted with bacteria suspensions shown to have concentrations of 10^6 to 10^9 organisms per ml. Virus culture supernatants are listed accordingly. The results of that study are summarized in Table 2.

Table 2: Cross reactivity with pathogenic microorganisms

Test germ	Origin	[OD450/620] mean value
Adenovirus	Supernatant of cell culture	0.032
Aeromonas hydrophila	Culture	0.023
Bacillus cereus	Culture	0.054
Bacteroides fragilis	Culture	0.034
Campylobacter coli	Culture	0.039
Campylobacter jejuni	Culture	0.031
Candida albicans	Culture	0.049
Citrobacter freundii	Culture	0.028
Clostridium difficile	Culture	0.032
Clostridium perfringens	Culture	0.031
Clostridium sordellii	Culture	0.033
Cryptosporidium muris	Culture	0.025
Cryptosporidium parvum	Culture	0.026
E. coli (O26:H-)	Culture	0.035
E. coli (O6)	Culture	0.030
E. coli (O157:H7)	Culture	0.039
Entamoeba histolytica	Culture	0.045
Enterobacter cloacae	Culture	0.028
Enterococcus faecalis	Culture	0.033
Giardia lamblia	Culture	0.032
Klebsiella oxytoca	Culture	0.035
Proteus vulgaris	Culture	0.035
Pseudomonas aeruginosa	Culture	0.035
Rotavirus	Supernatant of cell culture	0.029
Salmonella enteritidis	Culture	0.030
Salmonella typhimurium	Culture	0.033
Serratia liquefaciens	Culture	0.022
Shigella flexneri	Culture	0.024
Staphylococcus aureus	Culture	0.054
Staphylococcus epidermidis	Culture	0.035
Vibrio parahaemolyticus	Culture	0.030
Yersinia enterocolitica	Culture	0.031

13.3. Precision

The reproducibility of RIDASCREEN® Astrovirus ELISA was tested with six references representing the complete measurement range from negative to high positive. To determine the intra-assay reproducibility, 40 replicates of these references were assayed. The mean values and the coefficient of variation (CV) were determined for three lots of the kits. For the inter-assay reproducibility, references were assayed in duplicates for a total of 20 runs over several

days. The measurements were determined using three lots by three technicians. For inter-lot reproducibility results of all three lots were combined. The results of that study are shown in Table 3.

Table 3: Reproducibility and precision of the RIDASCREEN® Astrovirus 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	2.447	2.146	1.694	1.682	1.655	1.808	1.715
	CV (%)	**4.74 %**	**6.62 %**	**4.25 %**	**10.55 %**	**9.38 %**	**9.11 %**	**10.62 %**
2	MV	1.879	1.596	1.285	1.254	1.223	1.350	1.275
	CV (%)	**5.75 %**	**6.71 %**	**5.99 %**	**8.62 %**	**10.50 %**	**9.64 %**	**10.76 %**
3	MV	1.094	0.884	0.776	0.738	0.708	0.798	0.748
	CV (%)	**6.40 %**	**7.04 %**	**8.21 %**	**12.80 %**	**11.19 %**	**12.75 %**	**13.56 %**
4	MV	0.746	0.602	0.523	0.560	0.528	0.589	0.559
	CV (%)	**5.89 %**	**7.79 %**	**8.89 %**	**14.83 %**	**14.84 %**	**15.45 %**	**15.69 %**
5	MV	0.370	0.281	0.356	0.275	0.252	0.279	0.269
	CV (%)	**15.97 %**	**12.41 %**	**15.12 %**	**12.59 %**	**15.04 %**	**13.81 %**	**14.59 %**
6	MV	0.040	0.018	0.065	0.033	0.022	0.020	0.025
	CV (%)	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**

13.4 Analytical sensitivity

To determine the analytical sensitivity of RIDASCREEN® Astrovirus ELISA the LoB (Limit of Blank) has been established by 90 measurements of negative samples. Subsequently the LoD (Limit of Detection) has been determined by 30 measurements of an lysate of cells that had been infected by astroviruses. Results of these measurements are summarized in Table 4.

Table 4: Results of determination of analytical sensitivity of RIDASCREEN® Astrovirus ELISA

	MV [OD450/620]	ng/ml
LoB	0.027	-
LoD	-	8.5

14. Interfering substances

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

barium sulfate (18.5 % w/w), loperamide (0.02 % w/w), cyclamate/Saccharin (1.3 % v/w), human blood (5.0 % v/w), metronidazole (3.0 % v/w), diclofenac (0.1 % v/w).
Pepto-Bismol, mucins, stearic acid and palmitic acid may lead to reduction of extinctions.

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

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