DRG® Astrovirus Ag ELISA (EIA-4456)

IVD

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1 INTRODUCTION

Astrovirus was first described in 1975 and named according to its star-shaped structure visible under the electron microscope.
Astrovirus belongs to the family Astroviridae. Human Astroviruses are subdivided into 7 serotypes (1).
Together with Rotavirus and Adenovirus Astrovirus is one of the most common causes of non-bacterial gastroenteritis in children under 5 years of age all over the world. Thus 80% of children between 5 and 10 years of age are anti-Astrovirus-antibody positive. Astrovirus caused gastroenteritis in adults and nosocomial infections are observed as well (2).
The course of the disease is usually self-limiting and of short duration. After an incubation time of 1-2 days a 1-4 days lasting gastroenteritis develops accompanied by vomiting, diarrhea, fever and abdominal pain finally causing dehydration. Although occurring all over the year Astrovirus infections are mainly observed during the winter months (3,4).
Astrovirus infections are spread via fecal-oral transmission from person to person or via contaminated things or food. Infected persons excrete high amounts of Astrovirus particles with their feces (1,2).
The detection of Astrovirus may be performed by electron microscopy or by molecular biology techniques such as polymerase chain reaction (PCR). Meanwhile immunological methods like enzyme immunoassay have established as preferential methods for routine laboratory diagnosis since these methods are fast, economical and automation is possible (1).

2 INTENDED USE

DRG® Astrovirus Ag ELISA is an in-vitro-diagnostic device for direct detection of Astrovirus in fecal samples.

3 PRINCIPLE OF THE TEST

DRG® Astrovirus AG ELISA is a fast enzymometric one-step immunoassay on the basis of polyclonal and monoclonal antibodies.
Diluted stool specimens and horseradish peroxidase (HRP) labeled monoclonal anti-Astrovirus-antibodies are dispensed simultaneously into the wells of a microtitration plate coated with polyclonal anti-Astrovirus-antibodies.
After an incubation time of 60 min at room temperature (RT) unbound components are removed by a washing step.
HRP converts the subsequently added colorless substrate solution of 3,3',5,5'-Tetramethylbenzidine (TMB) within a 10 min reaction time at room temperature protected from light into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells turning the solution from blue to yellow.
The optical density (OD) of the solution read at 450/620 nm is directly proportional to the specifically bound amount of Astrovirus.

4 PREPARATION AND STORAGE OF SAMPLES

4.1 Collection and storage

Stool samples should be stored at 2-8°C immediately after collection and processed within 48 hours.
Longer storage is possible at -20°C. Repeated freezing and thawing of samples should be avoided.

4.2 Preparation

Quickly thaw frozen samples; warm samples to room temperature and mix well.

Pipette 1000 µl of *Sample Diluent* into a clean tube.

Using a disposable stirring rod transfer about 100 mg (diameter about 2-3 mm) of feces if solid or pipette 100 µl if liquid into the tube and suspend thoroughly.

If necessary, sediment floating particles by a centrifugation step.

5 TEST COMPONENTS FOR 96 WELLS

Microtiter wells	Microtitration plate, 12 single breakable 8-well strips (in all 96 wells) coated with polyclonal anti-Astrovirus-antibodies (rabbit)	1 vacuum sealed with desiccant
Wash Buffer 10X	Wash buffer, 10-fold for 1000 ml solution	100 ml concentrate white cap
Sample Diluent	Sample diluent	100 ml ready to use black cap
Positive Control	Positive control Astrovirus ELISA reactive sample	1.5 ml ready to use red cap
Negative Control	Negative control Astrovirus negative sample	1.5 ml ready to use green cap
HRP Conjugate	HRP-conjugate; HRP-labelled, monoclonal anti-Astrovirus-antibodies (mouse)	12 ml ready to use brown cap
TMB Substrate Solution	Substrate 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml ready to use blue cap
Stop Solution	Stop solution 0.25 M Sulphuric acid	15 ml ready to use yellow cap

6 MATERIALS REQUIRED BUT NOT PROVIDED

- micropipettes
- multi-channel pipette or multi-pipette
- reagent container for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water
- glassware

– tubes (2 ml) for sample preparation

7 PREPARATION AND STORAGE OF REAGENTS

7.1 Kit size and expiry

One kit is designed for 96 determinations.
The expiry date of each component is reported on its respective label, of the complete kit on the outer box label.
Upon receipt, all test components have to be kept at 2-8°C, preferably in the original kit box.
After opening all kit components are stable for at least 2 months, provided proper storage.

7.2 Reagent preparation

Allow all components to reach room temperature prior to use in the assay.

The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with distilled or de-ionized water.
For Example: 10 ml *Wash Buffer* concentrate + 90 ml distilled water.
This ready to use wash buffer solution is stable for at least 30 days when stored at 2-8°C.

Make sure that the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every single wash cycle!

Avoid light exposure of the TMB substrate solution!

8 ASSAY PROCEDURE

- Dilute samples with *Sample Diluent* 1 + 10, e.g. 100 mg or 100 µl stool + 1.0 ml sample diluent
- Avoid any time shift during dispensing of reagents and samples.

8.1 Working steps

1. Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.
2. Dispense **2 drops (or 75 µl)** ***HRP Conjugate*** and
3. **2 drops (or 75 µl)** ***Positive Control***
   Negative Control
   50 µl diluted specimen, mix gently.
4. Cover plate and incubate for **60 min** at RT.
5. Decant, then wash each well **5x** with **300 µl** wash solution (diluted from 2) and tap dry onto absorbent paper.
6. Dispense **2 drops (or 75 µl)** ***TMB Substrate Solution*** per well.
7. Incubate for **10 min** at RT protected from light.
8. Dispense **2 drops (or 75 µl)** ***Stop Solution***, mix gently.
9. Read OD at **450 nm** (reference filter 620 or 690 nm) with a microplate reader within 30 min after reaction stop.

9 RESULT INTERPRETATION

Qualitative evaluation

Cut-off determination: **OD negative control + 0.10**

Samples with OD values equal with or higher than the cut-off are considered **positive**, samples with OD values below the cut-off are considered **negative** for Astrovirus antigen.

10 REFERENCE VALUES

Astrovirus Antigen

Negative	< Cut-off
Positive	≥ Cut-off

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

10.1 Test validity

The test run is valid if:

- the mean OD of the negative control is ≤ 0.15
- the mean OD of the positive control is ≥ 1.00

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

10.2 Limitations of the procedure

There is no correlation between measured absorbance and seriousness of the infection.

It is also not allowed to correlate absorbances of the samples with that of the positive control.

Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions, not sufficiently homogenized samples or solid particles after centrifugation of the suspension can cause false negative as well as false positive results. Fermented samples with pH values below 5 after resuspension may produce false negative results.

A negative test result not necessarily excludes an Astrovirus infection.

Inhomogeneous virus distribution in the sample can cause false negative results.

The investigation of samples that were taken beyond the acute phase of the disease can cause false negative results, because the number of virus particles has decreased under the detection limit of the test. It is therefore recommended to take samples within the acute phase of the disease where a maximum number of excreted virus particles is to be expected.

A final interpretation of the test results should consider clinical findings as well.

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11 PERFORMANCE CHARACTERISTICS

11.1 Precision

Intra-assay coefficient of variation (CV) in the *DRG® Astrovirus Ag ELISA* calculated from 8fold determination of samples:

Sample	Mean OD	Standard deviation	CV (%)
1	1.667	0.148	8.9
2	0.994	0.063	6.4
3	0.443	0.027	6.1
4	0.185	0.018	9.8

Inter-assay coefficient of variation (CV) in the *DRG® Astrovirus Ag ELISA* from 6 different test runs from 8fold determination of samples:

Sample	Mean OD	Standard deviation	CV (%)
1	1.853	0.071	3.8
2	1.019	0.059	5.8
3	0.583	0.069	11.9
4	0.350	0.034	9.7

11.2 Lower detection limit

The lower detection limit of Astrovirus antigen in the *DRG® Astrovirus Ag ELISA* was determined by titration of purified Astrovirus-antigen.
Lower detection limit: 6 ng/ml.

11.3 Specificity and Sensitivity

A total of 98 stool samples was tested in parallel with the *DRG® Astrovirus Ag ELISA* and another commercially available ELISA.

	comparative ELISA positive	comparative ELISA negative
DRG® ELISA positive	49	0
DRG® ELISA negative	2	47

Specificity: 100 %
Sensitivity: 96 %

11.4 Cross reactivity

Rotavirus positive (n=16) and Adenovirus positive (n=6) stool samples did not cross react in the *DRG® Astrovirus Ag ELISA.*

12 COMMON ADVICES AND PRECAUTIONS

This kit is for in vitro use only.

Follow the working instructions carefully. The kit should be performed by trained technical staff only.

The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.

Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.

Do not use reagents from other manufacturers.

Avoid time shift during dispensing of reagents.

All reagents should be kept at 2-8°C before use.

Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucous membranes.

Handle all components and all patient samples as if potentially hazardous.

Since the kit contains potentially hazardous materials, the following precautions should generally be observed:
- Do not smoke, eat or drink while handling kit material,
- Always use protective gloves,
- Never pipette material by mouth,
- Note safety precautions of the single test components.

References:

1. Rohwedder, A. (2000): "Virale Gastroenteritiden, Erreger und Diagnostik", Mikrobiologe, 10. Jg. P.121-126.
2. Palombo, E. A. and Bishop, R. F. (1996): "Annual Incidence, Serotype Distribution and Genetic Diversity of Human Astrovirus Isolates from Hospitalized Children in Melbourne, Australia"; Journal of Clinical Microbiology, Vol. 34, No. 7, p. 1750-1753.
3. Cukor, G. and Blacklow, N. R. (1984): "Human Viral Gastroenteritis", Microbiological Reviews, June, Vol. 48 No. 2, p. 157-179.
4. Gaggero, A.; O´Ryan, M. et al. (1998): "Prevalence of Astrovirus Infection among Chilean Children with Acute Gastroenteritis", Journal of Clinical Microbiology, Vol. 36, No. 12, p. 3691-3693.