



INSTRUCTION MANUAL

REF 6008

March 11th, 2005

INTENDED USE

Astrovirus Antigen is used for the qualitative determination of Astrovirus antigen in fecal specimens.

Astrovirus was firstly 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 faecal-oral transmission from person to person or via contaminated things or food. Infected persons excrete high amounts of Astrovirus particles with their faeces (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).

1. Rohwedder A.: Virale Gastroenteritiden, Erreger und Diagnostik, Mikrobiologie 2000, 10, 121-126
2. Palombo E.A., Bishop R.F.: Annual Incidence, Serotype Distribution and Genetic Diversity of Human Astrovirus Isolates from Hospitalized Children in Melbourne, Australia, J. Clin. Microbiol. 1996, 34, 1750-1753
3. Cukor G., Blacklow N.R.: Human Viral Gastroenteritis, Microbiol. Rev. 1984, 48, 157-179
4. Gaggero A., O’Ryan M): Prevalence of Astrovirus Infection among Chilean Children with Acute Gastroenteritis, J. Clin. Microbiol. 1998, 36, 3691-3693

PRINCIPLE OF THE TEST

Astrovirus Antigen is a fast enzymometric one-step immunoassay for the qualitative determination of Astrovirus antigen employing a solid phase immobilized polyclonal antibody and a monoclonal antibodies conjugated to horseradish peroxidase.

Astrovirus antigens of specimens and the positive control react simultaneously with anti-Astrovirus-IgG conjugated to horseradish peroxidase (HRP) and with anti-Astrovirus antibodies coated on the solid phase of the microplate. After an incubation period of 60 min at room temperature (RT), unbound components are separated from the solid-phase immune complexes formed by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 10 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution read at 450 nm is directly proportional to the amount of Astrovirus antigen bound. For optimal results a reference filter (620 nm wavelength) should be used. Considering the cut off value results are interpreted as positive or negative.

Astrovirus Antigen

- 96 determinations -



IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of Astrovirus Antigen in fecal specimens

REF	Catalogue number	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk



GA GENERIC ASSAYS GmbH

Ludwig-Erhard-Ring 3

15827 Dahlewitz, Germany

Telephone: +49 (0) 33708 – 9286-0
Fax: +49 (0) 33708 – 9286-50

www.genericassays.com

PATIENT SAMPLES

Specimen collection and storage

Stool samples should be stored at 2...8°C immediately after collection and processed within 48 hours. Long-term storage requires - 20 °C. Repeated freezing and thawing of samples should be avoided. Fecal samples should be collected into containers that do not contain preservatives, metal ions or oxidizing agents.

Preparation before use

Allow frozen or refrigerated fecal samples to reach room temperature prior to assay. Take care to agitate samples gently in order to ensure homogeneity.

The Astrovirus Antigen ELISA is intended for the detection of Astrovirus in 1+10 externally diluted stool specimens. Using a disposable stirring rod transfer about 100 mg (diameter about 2-3 mm) of faeces if solid or pipette 100 µl if liquid into 1.0 ml sample diluent (C) and suspend thoroughly. Rectal swabs should be suspended in 1 ml sample diluent by pressing the swab to the inner wall of the tube several times (make sure that the sample volume is sufficient). Mix samples thoroughly, e. g. on a vortex. If necessary sediment floating particles of the homogenous suspension by centrifugation.

TEST COMPONENTS FOR 96 WELLS

A	Microtiter plate , 12 breakable strips per 8 wells coated with polyclonal antibodies to Astrovirus antigen (rabbit)	1 vacuum sealed with desiccant
Ag 96		
B	Concentrated wash buffer sufficient for 1000 ml solution	100 ml concentrate capped white
BUF WASH 10x		
C	Sample diluent	100 ml ready for use capped black
DIL		
D	Conjugate containing monoclonal antibodies to Astrovirus antigen (murine) coupled with HRP	12 ml ready for use capped brown
CONJ		
E	Substrate 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
SOLN TMB		
F	Stop solution 0.25 sulfuric acid	15 ml ready for use capped yellow
H2SO4 0.25 M		
P	Positive control Astrovirus antigen reactive specimen (inactivated)	1.5 ml ready for use capped red
CONTROL +		
N	Negative control Astrovirus antigen negative specimen	1.5 ml ready for use capped green
CONTROL -		

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

Size and storage

Astrovirus Antigen has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the Astrovirus Antigen 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.

Preparation before use

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

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate 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 with de-ionized or distilled water.

For example, dilute 5 ml of the concentrate with 45 ml of distilled water per strip. The wash solution prepared is stable at 2 - 8 °C up to 30 days.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- Dilute samples with sample diluent (C) 1 + 10 (w/v), e.g. 100 mg stool specimen + 1 ml sample diluent (C)
- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (20-25°C) before use. Mix gently without causing foam.
2. Dispense **two drops or 75 µl** conjugate (D) into all wells
3. Add
2 drops or 75 µl negative control (N)
2 drops or 75 µl positive control (P)
50 µl diluted samples into the respective wells
4. Seal plate, incubate **60 min** at room temperature (20-25°C).
5. Decant, then wash each well **five times** using **300 µl** wash solution (made of B).
6. Dispense **2 drops or 75 µl** of substrate (E) to each well.
7. Incubate **10 min protected from light** at room temperature (20-25°C).
8. Add **2 drops or 75 µl** of stop solution (F) to each well and mix gently.
9. Read the OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

DATA PROCESSING

Qualitative evaluation

Cut-off determination

OD of the negative control + 0.10 OD units

REFERENCE VALUES

Astrovirus antigen	
Negative	< Cut-off
Positive	≥ Cut-off

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.

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.

Example of typical assay results

wells	OD (a)	OD (b)	OD (mean)
Negative control	0.098	0.090	0.094
Positive control	1.516	1.534	1.525
Positive	≥ 0.094 + 0.100 = 0.194		
Specimen 1	1.218	1.186	1.202 – positive
Specimen 2	0.148	0.156	0.152 – negative

Test validity

The test run is valid if:

- the mean OD of the negative control is ≤ 0.150
- the mean OD of the positive control is ≥ 1.000

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.

Limitations of the method

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.

CHARACTERISTIC ASSAY DATA

Precision

Intra-assay coefficient of variation (CV) in the Astrovirus Antigen ELISA from 8fold determinations 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 Astrovirus Antigen ELISA in 6 different test runs from 8fold determinations 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

Lower detection limit

The lower detection limit of Astrovirus antigen in this ELISA was determined by titration of purified Astrovirus-antigen.

Lower detection limit: 6 ng/ml

Specificity and sensitivity

A total of 98 stool samples was investigated in parallel in the Astrovirus Antigen ELISA and in another commercially available ELISA.

	comparative ELISA positive	comparative ELISA negative
Adenovirus Antigen positive	49	0
Adenovirus Antigen negative	2	47

Specificity: 100 %

Sensitivity: 96 %

Cross reactivity

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

REMARKS:

INCUBATION SCHEME

Astrovirus Antigen (6008)

Dilute patients sample	100 mg sample + 1 ml sample diluent (C)
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1	Bring all reagents to room temperature (20-25°C)	
2	Dispense conjugate (D)	2 drops (or 75 µl)
3	Dispense Negative control (N) Positive control (P) 1 + 10 (w/v) prediluted samples	2 drops (or 75 µl) 2 drops (or 75 µl) 50 µl
3	Seal plate and incubate	60 min., room temperature (20-25°C)
4	Wash	Decant, 5 x 300 µl wash solution (made of B)
5	Pipette substrate (E)	2 drops (or 75 µl)
6	Incubate protected from light	10 min., room temperature (20-25°C)
7	Pipette stop solution (F)	2 drops (or 75 µl)
8	Read at 450 nm against 620 (690) nm within 30 min.	

SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. 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.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- 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 mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
 - Do not smoke, eat or drink while handling kit material,
 - Always use protective gloves,
 - Never pipette material by mouth,
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.