



# INSTRUCTION MANUAL

REF 6001

February 25<sup>th</sup>, 2004

## Rotavirus Antigen

- 96 determinations -



IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of Rotavirus Antigen in fecal specimens

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



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### INTENDED USE

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

Group A Rotaviruses are the most common cause of non-bacterial gastroenteritis in children aged between 4 months and 3 years (1-5); Rotaviruses are excreted into the intestine in large amounts ( $10^9$  -  $10^{11}$  virus particles per g feces). Nosocomial infections therefore cause problems especially on baby wards and in children's hospitals (3). Rotavirus infections can also be the reason for travelers diarrhea in adults and have been detected in stool specimens of asymptomatic carriers as well (1).

Enteral Rotavirus infections are transmitted via the fecal-oral route from person to person or contaminated objects can be the source of infection. In temperate climates, Rotavirus infections are mainly observed in the winter months, in contrast to bacterial diarrheas, that are most commonly detected during summer and autumn (1).

Because virus culture on primary monkey kidney cells or permanent cell lines is time consuming, these methods are of no diagnostic relevance. The golden standard for the diagnosis of Rotavirus infections is direct virus detection by electron microscopy (1, 2).

Meanwhile antigen detection methods based on immunological techniques as agglutination tests or enzyme immunoassays using polyclonal or monoclonal antibodies against the group specific antigen A (VP 6) have been developed (1-5).

Rotavirus detection from fecal samples should be carried out within the first 3 days after onset of symptoms and is successful in more than 90% of the cases. A negative test result does not necessarily exclude a Rotavirus infection. Stool specimens taken at the wrong time (after the 8. or 9. day after onset of symptoms a virus detection is rarely successful) or the stool consistency can cause false negative results.

1. Böthig, B. und Diedrich, S. (1996): „Rotaviren“. Diagnostische Bibliothek Band 1 Virusdiagnostik, Hrsg. Tomas Porstmann, Blackwell Wissenschafts-Verlag Berlin, Wien 1996, S. 441-451
2. Grauballe, B.F. et al. (1981): „Optimized Enzyme-Linked Immunosorbent Assay for Detection of Human and Bovine Rotavirus in Stools: Comparison With Electron-Microscopy, Immunoelectro-Osmophoresis, and Fluorescent Antibody Techniques.“ Journal of Medical Virology 7: 29-40.
3. Coulson, B.S. and I.H. Holmes (1984): „An Improved Enzyme-Linked Immunosorbent Assay For The Detection Of Rotavirus In Faeces of Neonates.“ Journal of Virological Methods, 8: 165-179
4. Cukor G. et al. (1984): „Detection of Rotavirus in Human Stools by Using Monoclonal Antibody.“ Journal of Clinical Microbiology 19: 888-892
5. Cukor G. and N.R. Blacklow (1984): „Human Viral Gastroenteritis.“ Microbiological Reviews 48, No.2, 157-179

### PRINCIPLE OF THE TEST

Rotavirus Antigen is a fast enzymometric one-step immunoassay for the qualitative determination of Rotavirus antigen employing a solid phase immobilized polyclonal antibody (sheep) and a murine monoclonal antibodies conjugated to horseradish peroxidase. Both antibodies are directed against the group specific VP6 antigen of group A rotaviruses.

Rotavirus antigens of specimens and the positive control react with anti-Rotavirus-IgG conjugated to horseradish peroxidase (HRP) and simultaneously with anti-Rotavirus 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 Rotavirus 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.

## PATIENT SAMPLES

### Specimen collection and storage

The Rotavirus Antigen ELISA is intended for the detection of Rotavirus in 1:11 externally diluted stool specimens (100 mg stool in 1.0 ml sample diluent (C)). 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 in a micro-centrifuge (e. g. Eppendorf) for 1 minute at maximum speed. 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 storage time at 2-8°C should not exceed 48 hours. Long-term storage requires - 20 °C. Repeated freezing and thawing of samples should be avoided.

## TEST COMPONENTS FOR 96 WELLS

<b>A</b>	<b>Microtiter plate</b> , 12 breakable strips per 8 wells coated with polyclonal antibodies to Rotavirus antigen (sheep)	1	vacuum sealed with desiccant
<b>Ag</b>	<b>96</b>		
<b>B</b>	<b>Concentrated wash buffer</b> sufficient for 1000 ml solution	100 ml	concentrate capped white
<b>BUF</b>			
<b>WASH</b>	<b>10x</b>		
<b>C</b>	<b>Sample diluent</b>	100 ml	ready for use capped black
<b>DIL</b>			
<b>D</b>	<b>Conjugate</b> containing anti-Rotavirus antigen-IgG- (murine) coupled with HRP	12 ml	ready for use capped brown
<b>CONJ</b>			
<b>E</b>	<b>Substrate</b> 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml	ready for use capped blue
<b>SOLN</b>			
<b>TMB</b>			
<b>F</b>	<b>Stop solution</b> 0.25 sulfuric acid	15 ml	ready for use capped yellow
<b>H2SO4</b>	<b>0.25 M</b>		
<b>P</b>	<b>Positive control</b> Rotavirus antigen SA 11 (inactivated)	1.5 ml	ready for use capped red
<b>CONTROL</b>	<b>+</b>		
<b>N</b>	<b>Negative control</b> Rotavirus antigen negative specimen	1.5 ml	ready for use capped green
<b>CONTROL</b>	<b>-</b>		

### Materials required but not provided

- micropipettes
- multi-channel pipette or multi-pipette trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- distilled or de-ionized water
- glassware

### Size and storage

Rotavirus 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 Rotavirus 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 + 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** conjugate (D) into the respective wells
3. Dispense  
**2 drops** negative control (N)  
**2 drops** positive control (P)  
**50 µl** diluted samples
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. Add **2 drops** of substrate (E) to each well.
7. Incubate **10 min protected from light** at room temperature (20-25°C).
8. Add **2 drops** 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 + 0.2 OD units

## REFERENCE VALUES

Rotavirus antigen	
Negative	$\leq$ cut-off
Positive	$>$ cut-off

### Example of typical assay results

wells	OD (a)	OD (b)	OD (mean)
Negative control	0.098	0.090	0.094
Positive control	2.516	2.534	2.525
Positive	$> 0.094 + 0.200$		$= 0.294$
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  $\leq 0.20$
- the mean OD of the positive control is  $\geq 1.20$

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

Cross contaminations of the kit reagents and samples can cause false results. Samples should not be collected into containers with preservatives, metal ions or oxidizing agents. Samples should be stored at 2-8°C for a maximum time of 48 h before testing.

A negative test result not necessarily excludes a Rotavirus 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 (up to 3 days after onset of symptoms) where the number of excreted virus particles is at its height. Clinical symptoms have to be considered for a final interpretation of the test results.

## CHARACTERISTIC ASSAY DATA

### Precision

Intra-assay coefficient of variation (c. v.) in the Rotavirus Antigen ELISA calculated from 12fold determinations of the samples:

sample	OD mean	SD	c. v. (%)
I.	0.463	0.024	5.26
II.	0.620	0.040	6.38
III.	1.208	0.078	6.50
IV.	1.841	0.137	7.45

Inter-assay coefficient of variation (c. v.) in the Rotavirus Antigen ELISA in 10 different test runs calculated from 3fold determinations of the samples:

sample	OD mean	SD	c. v. (%)
I.	0.409	0.019	4.58
II.	0.968	0.074	7.69
III.	1.647	0.122	7.38
IV.	2.720	0.128	4.71

### Lower detection limit

The lower detection limit of Rotavirus antigen in the Rotavirus Antigen ELISA was determined by titration of purified Rotavirus antigen SA-11 in comparison with another commercially available ELISA. Lower detection limit of Rotavirus antigen in the Rotavirus antigen ELISA:  $< 10$  ng/ml SA-11 (corresponding to  $10^6$  virus particles/g feces).

### Clinical evaluation

A total of 330 stool specimens were tested in parallel with Rotavirus Antigen ELISA and another commercially available ELISA.

	Competitor ELISA	
	Positive	Negative
Rotavirus Antigen ELISA	84	2
	5	239

Specificity: 99.2 %  
Sensitivity: 94.4 %

### REMARKS:

## INCUBATION SCHEME

# Rotavirus Antigen (6001)

<b>Dilute patients sample</b>	<b>100 mg sample + 1 ml sample diluent (C)</b>
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1	<b>Bring all reagents to room temperature (20-25°C)</b>	
2	Dispense conjugate (D)	2 drops
3	Dispense	2 drops
	Negative control (N)	2 drops
	Positive control (P)	2 drops
	1 + 10 (w/v) prediluted samples	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
6	Incubate protected from light	10 min., room temperature (20-25°C)
7	Pipette stop solution (F)	2 drops
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.