Instructions For Use

Serazym® Rotavirus

Enzyme immunoassay for detection of *Rotavirus* in faecal samples

REF E-020 Σ 96 REF E-020-A2 Σ 2x 96 IVD *In-vitro*- diagnostic medical device CE

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Introduction

Group A Rotaviruses are the most common cause of non-bacterial gastroenteritis in children aged between 4 months and 3 years (1 - 5). Rotavirus is excreted into the intestine in large amounts (10^9 – 10^{11} virus particles per g faeces). Nosocomial infections therefore cause problems especially on baby wards and in childrens hospitals (3).
Rotavirus may also be responsible for travellers diarrhea in adults and have been detected in stool specimens of asymptomatic carriers as well (1). Rotavirus is spread by faecal-oral transmission from person to person or via contaminated staff. In temperate climates Rotavirus infections are mainly observed during the winter months (1).
Since virus culture on primary monkey kidney cells or permanent cell lines is time-consuming, these methods are of no diagnostic relevance. The golden standard is direct virus detection by electron microscopy (1, 2). Meanwhile antigen detection methods based on immunological techniques like agglutination tests or enzyme immunoassays using polyclonal or monoclonal antibodies against the group A specific antigen (VP-6) have been established (1 - 5).

References:

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,p. 157-179.

Intended Use

The *Serazym*® Rotavirus is an *in-vitro*-diagnostic medical device for direct detection of Rotavirus in faecal samples.

Principle Of The Test

Serazym® Rotavirus is a one-step enzyme immunoassay on the basis of polyclonal antibodies to the group specific VP-6 antigen, the major protein of group A Rotaviruses. Diluted stool specimens and horseradish peroxidase (HRP) labelled polyclonal anti-Rotavirus-antibodies are dispensed simultaneously into the wells of a microtitration plate coated with polyclonal anti-Rotavirus antibodies. After an incubation time of 60 min at room temperature unbound components are removed by a washing step. HRP converts the subsequently added colourless substrate solution of 3,3',5,5'-Tetramethylbenzidine (TMB) within a 10 min reaction time 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 Rotavirus. Considering the cut-off value results are interpreted as positive or negative.

Test Components

			For 96 Wells	For 2x 96 Wells
1	WELLS	**Microtitration plate** coated with polyclonal anti-Rotavirus-antibodies (sheep)	12 single breakable 8-well strips colour coding dark blue vacuum-sealed with desiccant	2x 12 single breakable 8-well strips colour coding dark blue vacuum-sealed with desiccant
2	WASHBUF CONC 10x	**Wash buffer** 10-fold	100 ml concentrate for 1000 ml solution white cap	2x 100 ml concentrate for 2x 1000 ml solution white cap
3	DIL	**Sample diluent**	100 ml · ready to use coloured yellow black cap	2x 100 ml · ready to use coloured yellow black cap
4	CONTROL +	**Positive control** Rotavirus reactive sample	1.5 ml · ready to use coloured blue red cap	3.0 ml · ready to use coloured blue red cap
5	CONTROL –	**Negative control** Rotavirus negative sample	1.5 ml · ready to use coloured blue green cap	3.0 ml · ready to use coloured blue green cap
6	CONJ HRP	**HRP-conjugate** HRP-labelled, polyclonal anti-Rotavirus-antibodies (rabbit)	12 ml · ready to use coloured green brown cap	24 ml · ready to use coloured green brown cap
7	SUBSTR TMB	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml · ready to use blue cap	28 ml · ready to use blue cap
8	STOP	**Stop solution** 0.25 M sulphuric acid	15 ml · ready to use yellow cap	28 ml · ready to use yellow cap

Preparation And Storage Of Samples

Collection and storage

Stool samples should be stored at 2…8°C immediately after collection and processed within 72 hours. Longer storage is possible at -20°C. Repeated freezing and thawing of samples should be avoided. Stool samples already diluted with the *Serazym*® sample diluent can be stored for up to 72 h at 2…8 °C before testing in the ELISA.

Preparation

Quickly thaw frozen samples. Warm samples to room temperature and mix well.
The *Serazym*® Rotavirus can be performed with 1 : 6 or 1 : 11 diluted specimens. In case of additional testing of the same sample in the *Serazym*® Norovirus, the *Serazym*® Campylobacter or the *Serazym*® Clostridium difficile Toxin A+B the 1 : 6 dilution is recommended.

Preparation of a 1 : 11 sample dilution:
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 faeces if solid or pipette 100 µl if liquid into the tube and suspend thoroughly. If necessary, sediment floating particles by a centrifugation step with a micro centrifuge for one min at maximum speed.

Preparation of a 1 : 6 sample dilution:
Pipette 1000 µl of sample diluent into a clean tube. Using a disposable stirring rod transfer about 200 mg (diameter about 4 - 6 mm) of faeces if solid or pipette 200 µl if liquid into the tube and suspend thoroughly. If necessary, sediment floating particles by a centrifugation step with a micro centrifuge for one min at maximum speed.

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 of 450 nm for measurement and ≥ 620 nm for reference · distilled or deionized water · glassware · tubes (2 ml) for sample preparation

Preparation And Storage Of Reagents

Kit size and expiry
One kit is designed for 1x 96 or 2x 96 determinations. The expiry date of each component is reported on its respective label, that 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. The ready to use wash solution can be used for at least 1 month when stored at 2…8°C.

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 10-fold concentrated wash buffer 1 + 9 with distilled or deionized water.
For Example: 10 ml wash buffer concentrate *(2)* + 90 ml distilled or deionized water.

Assay Procedure

Dilute samples with sample diluent *(3)* 1 : 11 or 1 : 6, e.g. 100 mg or 100 µl stool + 1.0 ml (1 : 11) sample diluent *(3)* or 200 mg or 200 µl stool + 1.0 ml (1 : 6) sample diluent *(3)*.
Avoid any time shift during dispensing of reagents and samples.
Make sure 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!

Working steps

1. Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.
2. Dispense 2 drops (or 75 µl) **CONJ HRP** HRP-conjugate *(6)* per well and
3. Pipette: 75 µl **CONTROL +** positive control *(4)*
   75 µl **CONTROL –** negative control *(5)*
   50 µl **diluted sample**, 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 if necessary.
6. Dispense 2 drops (or 75 µl) **SUBSTR TMB** substrate *(7)* per well.
7. Incubate for 10 min at RT protected from light.
8. Dispense 2 drops (or 75 µl) **STOP** stop solution *(8)* per well, mix gently.
9. Read OD at 450 nm / ≥ 620 nm with a microplate reader within 30 min after reaction stop.

Result Interpretation

Qualitative evaluation
Cut-off determination: OD negative control + 0.20
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 Rotavirus antigen.

Reference Values

Serazym® Rotavirus	
Positive	≥ Cut-off
Negative	< 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 mentioned reference values provide a guide only to values which might be expected.

Test validity
The test run is valid if:

- the mean OD of the negative control is ≤ 0.20 (manual performance)
  ≤ 0.30 (automatic performance)
- the mean OD of the positive control is ≥ 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 procedure

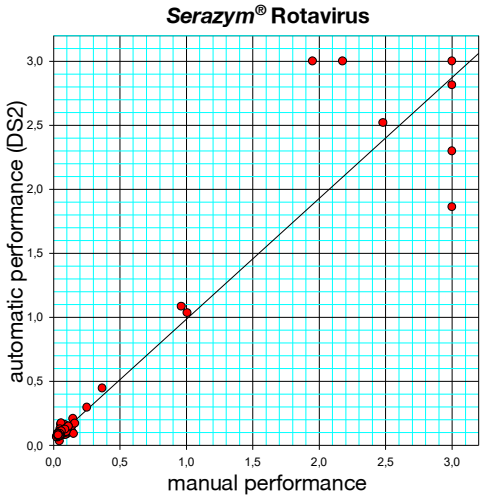
There is no correlation between measured absorbance and seriousness of the infection. It is also not allowed to correlate absorbances of the samples with that of the positive control. Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions, not sufficiently homogenized samples or solid particles after centrifugation of the suspension can cause false negative as well as false positive results. 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 where a maximum number of excreted virus particles are to be expected. Faecal samples from vaccinated children may contain vaccine virus causing positive ELISA results. A final interpretation of the test results should consider clinical findings as well.

Automatic Processing

Performing the *Serazym*® Rotavirus on fully automated microplate processors (e.g. DS2, DSX) may cause elevated absorbances in comparison to the manual procedure due to individual differences concerning wash procedures and general technical specifications of the equipment. In these cases a maximum value of 0.3 absorbance units is permissible for the negative control. It is recommended to use a wash procedure including 10 seconds soak time per strip and wash step followed by a wash step with distilled or deionized water with 10 seconds of soak time after the final wash step of each wash cycle. If necessary, the number of washing steps can be enhanced from 5x to 7x - 8x.

Correlation: manual – automatic processing

A panel of 133 stool specimens was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with r = 0.96.



Performance Characteristics

Precision

Intra-assay coefficient of variation (CV) in the *Serazym*® Rotavirus from 12-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	1.841	0.137	7.45
2	1.208	0.078	6.50
3	0.620	0.040	6.38
4	0.463	0.024	5.26

Inter-assay coefficient of variation (CV) in the *Serazym*® Rotavirus in 10 different test runs from 3-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	2.720	0.128	4.71
2	1.647	0.122	7.38
3	0.968	0.074	7.69
4	0.409	0.019	4.58

Lower detection limit

The lower detection limit of *Rotavirus* antigen in the *Serazym*® Rotavirus was determined by titration of purified *Rotavirus* antigen SA-11: < 10 ng / ml corresponding to 10^6 virus particles / g faeces.

Specificity and sensitivity

A total of 488 stool samples were investigated in parallel in the *Serazym*® Rotavirus and in another commercially available ELISA.

	comparative ELISA positive	comparative ELISA negative
***Serazym*® ELISA positive**	246	0
***Serazym*® ELISA negative**	4	238

Specifity: 100% · Sensitivity: 98.4%

Cross reactivity

Stool samples positive for one of the subsequent pathogens have been tested with the *Serazym*® Rotavirus and showed no cross reactivity:
Adenovirus (n = 20), Astrovirus (n = 8), Norovirus (n = 31), *Clostridium difficile* (n = 11), *Campylobacter jejuni* (n = 7), *Campylobacter coli* (n = 1), *Salmonella enteritidis* (n = 18), *Giardia lamblia* (n = 1) and stool samples (n = 93) with detectable levels (> 10 µg / g) of haemoglobin.

Negative stool specimens have been spiked with ≥ 10^8 colony forming units of the following microorganisms and tested negative with the *Serazym*® ELISA (OD 450 / 620 nm < Cut-Off):

Aeromonas hydrophila	(ATCC 7966)	*Klebsiella pneumoniae*	(ATCC 13883)
Bacillus cereus	(ATCC 11778)	*Peptostreptococcus anaerobius*	(ATCC 27337)
Bacillus subtilis	(ATCC 6633)	*Proteus vulgaris*	(ATCC 8427)
Bacteroides fragilis	(ATCC 25285)	*Pseudomonas aeruginosa*	(ATCC 10145)
Candida albicans	(ATCC 10231)	*Salmonella enterica Serovar enteritidis*	(ATCC 13076)
Campylobacter coli	(ATCC 33559)	*Salmonella enterica Serovar typhimurium*	(ATCC 14028)
Campylobacter jejuni	(ATCC 33291)	*Shigella flexneri*	(ATCC 12022)
Citrobacter freundii	(ATCC 8090)	*Shigella sonnei*	(ATCC 25931)
Clostridium sordellii	(ATCC 9714)	*Staphylococcus aureus*	(ATCC 25923)
Enterobacter aerogenes	(ATCC 13048)	*Staphylococcus epidermidis*	(ATCC 12228)
Enterobacter cloacae	(ATCC 13047)	*Vibrio parahaemolyticus*	(ATCC 17802)
Enterococcus faecalis	(ATCC 29212)	*Vibrio cholerae*	clinical isolate
Escherichia coli	(ATCC 25922)	*Yersinia enterocolitica Serotyp 03, 09*	clinical isolates

Interference

None of the following substances added to positive and negative stool samples showed a significant impact on the test result:
Barium sulfate (5%), Buscopan® (2 mg/ml), Cyclamate (5%), Diclofenac (2 mg/ml), Hemoglobin human (5 mg/ml), Blood human (5%), Hylak® N (5%), Iberogast® (5%), Immodium® akut duo (0.2/12.5 mg/ml), Loperamide (0.2 mg/ml), Metronidazole (2 mg/ml), Mucin (5 mg/ml), Nexium® (2 mg/ml), Palmitic acid (20%), Pentofuryl® (2 mg/ml), Pepto-Bismol (1 mg/ml), Perenterol (2.5 mg/ml), Rennie® (8 mg/ml), Simagel® (2 mg/ml), Stearic acid (20%), Vancomycin (2 mg/ml).

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. Do not use reagents from damaged packages or bottles. The expiration dates stated on the respective labels are to be observed. **Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.**
The sample diluent, wash buffer, TMB/substrate solution and stop solution is universally applicable for the *Serazym*® stool ELISA Adenovirus (E-017), Rotavirus (E-020), Astrovirus (E-045), Norovirus (E-061), Clostridium difficile Toxin A+B (E-040), Clostridium difficile GDH (E-107), Campylobacter (E-093), H. pylori 2nd Gen. (E-114), Entamoeba histolytica (E-018), Cryptosporidium parvum (E-039), Giardia lamblia (E-038) and Giardia (E-106).
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 may contain biocides as preservative. Further information can be found in the safety data sheet. 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!

History of Changes

Version	Section	Modifications
2020-11-16	Common Advices and Precautions	Update
	History of Changes	Adding section “History of Changes “
	Test Components	Update

Incubation Scheme *Serazym*® Rotavirus (E-020)

1.		2 drops (or 75 µl)	CONJ HRP *(6)*
		+	
		pipette	
		75 µl	CONTROL + *(4)*
		75 µl	CONTROL – *(5)*
		50 µl	**diluted stool sample,** mix gently
		60 min	incubation (room temperature)
		5 x Wash	with wash solution
2.		2 drops (or 75 µl)	SUBSTR TMB *(7)*
		10 min	incubation (room temperature) protected from light
3.		2 drops (or 75 µl)	STOP *(8)*

Read OD at 450 / ≥ 620 nm