Instructions For Use

Serazym® Adenovirus

Enzyme immunoassay for detection of *Adenovirus* in faecal samples

REF E-017 Σ 96 REF E-017-A2 Σ 2x 96 IVD *In-vitro*-diagnostic medical device CE

Seramun Diagnostica GmbH · Spreenhagener Straße 1 · 15754 Heidesee · Germany · **www.seramun.com**
phone +49 (0) 33767 79110 · fax +49 (0) 33767 79199 · info@seramun.com

Introduction

Adenovirus is the causative agent of respiratory tract infections, conjunctivitis or enteritis. *Adenovirus* infections are spread via faecal-oral transmission or aerosols (1). Most infections are mild to moderate and do not last longer than one week. *Adenovirus* is responsible for 2 - 8% of respiratory tract infections and 7 - 17% of diarrheal diseases in children (2). Thirty percent of viral diarrheas in immunocompromised patients are caused by *Adenovirus* infections (1). The diagnosis of *Adenovirus* infections is preferably done by direct detection in faecal specimens or swabs. Due to the difficulty of *Adenovirus* culture on tissue or cell culture virus detection so far has been performed by electron microscopy. Meanwhile immunological methods like enzyme immunoassay (ELISA) have been developed for antigen detection (3). ELISA techniques are based on poly- and/or monoclonal antibodies to the protein hexon representing the major part of the virus capsid.

References:

1. Mentel, R. und Döhner, L. (1996): „Humane Adenoviren.“ Diagnostische Bibliothek Band 1 Virusdiagnostik, Hrsg. Tomas Porstmann, Blackwell Wissenschafts-Verlag Berlin, Wien 1996, S. 103-114
2. Schoenemann W. (1988): “Bedeutung von Adenovirusinfektionen im Säuglings- und Kleinkindesalter” Monatsschrift Kinderheilkunde 136: 680-685
3. August, M. J. and Warford, A. L. (1987): „Evaluation of a Commercial Monoclonal Antibody for Detection of Adenovirus Antigen.” Journal Of Clinical Microbiology, Vol. 25, No. 11: 2233-2235

Intended Use

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

Principle Of The Test

Serazym® Adenovirus is a one-step enzyme immunoassay on the basis of monoclonal antibodies against an epitope of the capsid protein hexon, common to all human pathogenic *Adenovirus* serotypes. Diluted stool specimens and horseradish peroxidase (HRP) labelled monoclonal anti-Adenovirus-antibodies are dispensed simultaneously into the wells of a microtitration plate coated with monoclonal anti-Adenovirus-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 colourless 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 *Adenovirus*. 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 monoclonal anti-Adenovirus-antibodies (mouse)	12 single breakable 8-well strips colour coding violet vacuum-sealed with desiccant	2x 12 single breakable 8-well strips colour coding violet 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** *Adenovirus* antigen Ad 41 (inactivated)	1.5 ml · ready to use coloured blue red cap	3.0 ml · ready to use coloured blue red cap
5	CONTROL –	**Negative control** *Adenovirus* 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, monoclonal anti-Adenovirus-antibodies (mouse)	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*® Adenovirus 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 30 days 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.
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 *Adenovirus* antigen.

Reference Values

Serazym® Adenovirus	
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:

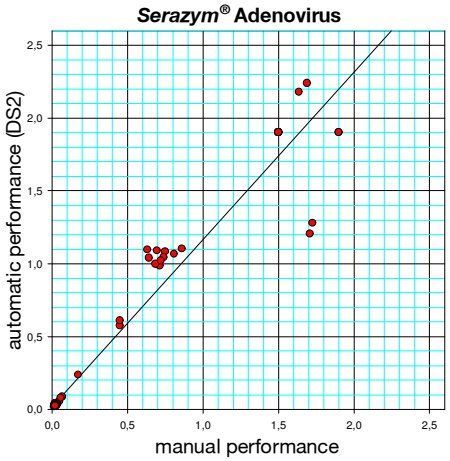
- the mean OD of the negative control is ≤ 0.20 (manual performance)
  ≤ 0.30 (automatic performance)
- the mean OD of the positive control is ≥ 1.20

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

Automatic Processing
Performing the *Serazym*® Adenovirus 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 110 stool specimens was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with r = 0.974.



Performance Characteristics

Precision

Intra-assay coefficient of variation (CV) in the *Serazym*® Adenovirus from 8-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	2.792	0.160	5.7
2	2.059	0.167	8.1
3	1.368	0.094	6.9
4	0.718	0.068	9.4

Inter-assay coefficient of variation (CV) in the *Serazym*® Adenovirus in 6 different test runs from 8-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	1.850	0.107	5.8
2	1.057	0.069	6.5
3	0.574	0.042	7.3
4	0.312	0.030	9.6

Lower detection limit

The lower detection limit of *Adenovirus* antigen in the *Serazym*® Adenovirus was determined by titration of purified *Adenovirus* antigen (hexon). Lower detection limit: 6 ng / ml

Specificity and sensitivity

A total of 330 stool samples were investigated in parallel in the *Serazym*® Adenovirus and in another commercially available ELISA.

	comparative ELISA positive	comparative ELISA negative
***Serazym*® ELISA positive**	55	1
***Serazym*® ELISA negative**	2	272

Specifity: 99.6% · Sensitivity: 96.5%

Cross reactivity

Stool samples positive for one of the subsequent pathogens have been tested with the *Serazym*® Adenovirus and showed no cross reactivity:
Rotavirus (n = 10), *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).

Negative stool specimens have been spiked with $\geq 10^8$ 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), Hemoglobine human (5 mg/ml), Blood human (1,25%), 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!

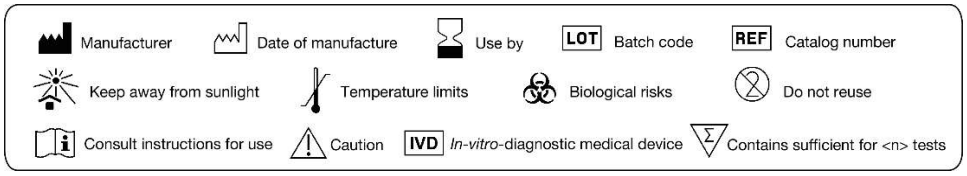
History of Changes

Version	Section	Modifications
2020-11-04	Common Advices and Precautions	Update
	History of Changes	Adding section “History of Changes “
	Test Components	Update

Incubation Scheme *Serazym*® Adenovirus (E-017)

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



Instructions For Use

Serazym® Astrovirus

Enzyme immunoassay for detection of *Astrovirus* in faecal samples

REF E-045 Σ 96 REF E-045-A2 Σ 2x 96 IVD *In-vitro*- diagnostic medical device CE

Seramun Diagnostica GmbH · Spreenhagener Straße 1 · 15754 Heidesee · Germany · **www.seramun.com**
phone +49 (0) 33767 79110 · fax +49 (0) 33767 79199 · info@seramun.com

Introduction

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 the 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, safe and automation is possible (1).

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.

Intended use

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

Principle of the test

Serazym® Astrovirus is a one-step enzyme immunoassay on the basis of polyclonal and monoclonal antibodies against *Astrovirus* antigens. Diluted stool specimens and horseradish peroxidase (HRP) labelled 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 colourless 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*.

Test components

			For 96 Wells	For 2x 96 Wells
1	WELLS	**Microtitration plate** coated with polyclonal anti-Astrovirus-antibodies (sheep)	12 single breakable 8-well strips colour coding light blue vacuum-sealed with desiccant	2x 12 single breakable 8-well strips colour coding light 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** *Astrovirus* 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** *Astrovirus* 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, monoclonal anti-Astrovirus-antibodies	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 48 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*® Astrovirus 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 30 days 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 + 0.10
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.

Reference values

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 ≤ 0.15 (manual test performance)
  ≤ 0.30 (automatic test performance)
- 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.

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 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 are to be expected. A final interpretation of the test results should consider clinical findings as well.

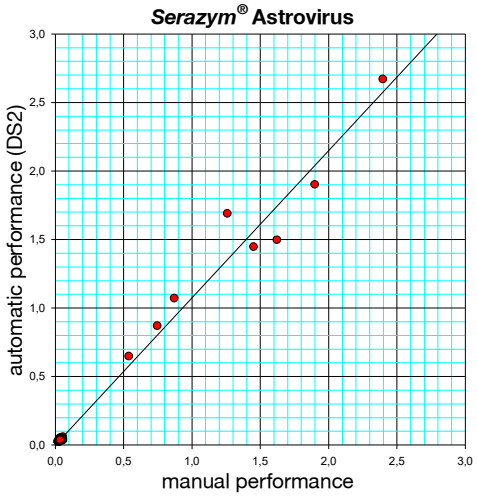
Automatic Processing

Performing the *Serazym*® Astrovirus 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.

Serazym® Astrovirus	
Positive	≥ Cut-off
Negative	< Cut-off

Correlation: manual – automatic processing

A panel of 96 stool specimens was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with r = 0.993.



Performance characteristics

Precision

Intra-assay coefficient of variation (CV) in the *Serazym*® Astrovirus from 8-fold determinations of samples:

sample	mean absorbance	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 *Serazym*® Astrovirus in 6 different test runs from 8-fold determinations of samples:

sample	mean absorbance	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 the *Serazym*® Astrovirus was determined by titration of purified *Astrovirus*-antigen. Lower detection limit: 6 ng / ml.

Specificity and sensitivity

A total of 98 stool samples were investigated in parallel in the *Serazym*® Astrovirus and in another commercially available ELISA.

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

Specificity: 100% Sensitivity: 96%

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

Negative stool specimens have been spiked with $\geq 10^8$ 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 sulphate (5%), Buscopan® (2 mg/ml), cyclamate (5%), Diclofenac (2 mg/ml), haemoglobine (5 mg/ml), Hylak® N (5%), Immodium® akut duo (0.2/12.5 mg/ml), Iberogast® (5%), 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%).

* The addition of 5% (v/v) Hylak® N (lactic acid containing preparation against digestive complaints) to Astrovirus positive stool suspensions may decrease OD values.

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 are 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!

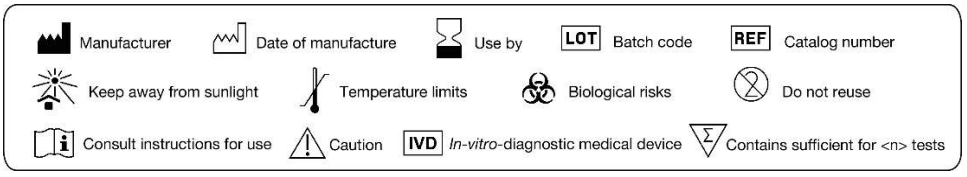
History of Changes

Version	Section	Modifications
2020-07-21	Intended use	Correction
	Test Components	Correction
	Assay Procedure	Update
	Common Advices and Precautions	Update
	History of Changes	New section “History of Changes“

Incubation scheme *Serazym*® Astrovirus (E-045)

1.		2 drops (or 75 µl)	CONJ HRP *(6)*
		+	
		pipette	
		75 µl	CONTROL + *(4)*
		75 µl	CONTROL – *(5)*
		50 µl	**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



Instructions For Use

Serazym® Norovirus

Enzyme immunoassay for detection of *Norovirus* in stool specimens

REF E-061 Σ 96 REF E-061-A2 Σ 2x 96 IVD *In-vitro*-diagnostic medical device CE

Seramun Diagnostica GmbH · Spreenhagener Straße 1 · 15754 Heidesee · Germany · **www.seramun.com**
phone +49 (0) 33767 79110 · fax +49 (0) 33767 79199 · info@seramun.com

Introduction

Noroviruses belong to the family *Caliciviridae*, single stranded RNA viruses of 30 - 40 nm in size characterized by a typical cup-shaped capsid. Within the genus *Norovirus* the two human pathogenic genogroups GGI and GGII have been identified. GGI and GGII strains can be further subclassified into at least 15 and 18 genotypes resp. The genetic heterogeneity of Noroviruses causes distinct capsid protein divergences between different genogroups (about 60%) as well as between different genotypes within one genogroup (about 20 - 30%). Since 1994 genotype GGII.4 is predominantly circulating. Noroviruses are very resistant to environmental conditions and highly contagious. The infection is transmitted by direct contact to already infected people either by faecal-oral transmission or by ingestion of aerosols from vomit or by contaminated food, drinking water or objects. After a short, 10 - 50 hours lasting incubation time fulminant diarrhea and often vomiting develop as the characteristic symptoms. The infection is usually self-limiting and symptoms disappear after 2 - 3 days. Norovirus infections are characterized by seasonal fluctuations with a climax during the winter months. They are considered as the most common cause of non-bacterial gastroenteritis outbreaks worldwide, but may also be responsible for single cases of viral gastroenteritis. The high sequence variability of the capsid proteins circumvents the production of protective antibodies and hampers diagnostic detection. Methods like PCR (usually as “Real time Reverse Transcriptase PCR – Rt RT-PCR) and enzyme immunoassay are commonly used for laboratory diagnosis.

References:

1. Venkataram, B.V. et al. (1999): "X-ray Crystallographic Structure of the Norwalk Virus Capsid" Science Vol 286: 287-290.
2. Künkel, U. und Schreier, E. (2002): "Caliciviren-Virale Auslöser akuter Gastroenteritiden" Bundesgesundheitsbl-Gesundheitsforsch-Gesundheitsschutz 45: 534-542.
3. Hansman, G.S. et al. (2006): "Genetic and antigenic diversity among noroviruses" Journal of General Virology 87:909-919.
4. Marshall, J.A. and Bruggink, L.D. (2006): "Laboratory Diagnosis of Norovirus" Clin. Lab. 52: 571-581
5. Tomoyuki Shiota et al. (2007): "Characterization of a Broadly Reactive Monoclonal Antibody against Norovirus Genogroups I and II: Recognition of a Novel Conformational Epitope" Journal of Virology, Vol. 81, No. 22, p.12298-12306.
6. Lindesmith, L.C. et al. 2008: „Mechanisms of GII.4 Norovirus Persistence in Human Populations" PLoS Medicine Vol 5 (2): 269-289

Intended Use

The *Serazym*® Norovirus is an *in-vitro*-diagnostic medical device for direct detection of *Norovirus* specific antigens in stool specimens.

Principle Of The Test

Norovirus antigens from stool specimens and the positive control react with polyclonal anti-Norovirus antibodies coated on the solid phase of the microplate. After incubation non-bound material is removed by a washing step. Subsequently bound antigens specifically react with horseradish peroxidase (HRP) labeled polyclonal anti-Norovirus antibodies during a second incubation period. Unbound conjugate is removed by a washing step. HRP converts the subsequently added colourless chromogenic substrate solution (TMB / H_2O_2) 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 / $\geq$ 620 nm is directly proportional to the specifically bound amount of *Norovirus* antigens. By means of the calculated cut-off value, results are interpreted either as positive or negative.

Test Components

			For 96 Wells	For 2x 96 Wells
1	WELLS	**Microtitration plate** coated with polyclonal anti-Norovirus antibodies (sheep)	12 single breakable 8-well strips colour coding silver vacuum-sealed with desiccant	2x 12 single breakable 8-well strips colour coding silver 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** Recombinant Norovirus capsid proteins	2.0 ml · ready to use coloured blue red cap	4.0 ml · ready to use coloured blue red cap
5	CONTROL –	**Negative control** Norovirus negative sample	2.0 ml · ready to use coloured blue green cap	4.0 ml · ready to use coloured blue green cap
6	CONJ HRP	**HRP-conjugate** HRP-labelled polyclonal anti-Norovirus antibodies (sheep)	15 ml · ready to use coloured green brown cap	25 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

Warm samples to room temperature and mix thoroughly. 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 spin down floating particles in 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 450 nm filter 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 one 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 : 6, e.g. 200 mg or 200 µl stool + 1.0 ml sample diluent *(3)* and mix thoroughly on a vortex.
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 direct 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. Pipette: 100 µl CONTROL + positive control *(4)*
   100 µl CONTROL – negative control *(5)*
   100 µl **diluted stool specimen.**
3. Cover plate and incubate for 60 min at RT.
4. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
5. Dispense 3 drops (or 100 µl) CONJ HRP HRP-conjugate *(6)* per well.
6. Cover plate and incubate for 30 min at RT.
7. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
8. Dispense 3 drops (or 100 µl) SUBSTR TMB substrate *(7)* per well.
9. Incubate for 10 min at RT protected from light.
10. Dispense 3 drops (or 100 µl) STOP stop solution *(8)* per well, mix gently.
11. 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 + 0.10
Samples with OD values higher than or equal to the cut-off value are considered positive, samples with OD values below the cut-off value are considered negative for *Norovirus* antigen.

Reference Values

Serazym® Norovirus	
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:

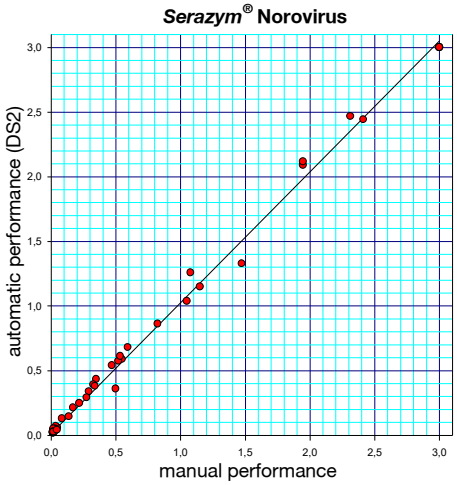
- the mean OD of the negative control is ≤ 0.20 (manual test performance)
  ≤ 0.30 (automatic test performance)
- the mean OD of the positive control is ≥ 1.20

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 *Norovirus* 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. Genetic recombination between different *Norovirus* strains may cause antigenic shift finally leading to the occurrence of virus variants that are not detected by ELISA. The overall interpretation of the ELISA results should always consider clinical findings.

Automatic Processing
Performing the *Serazym*® Norovirus on fully automated microplate processors (e.g. DS2 or 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 90 stool specimens was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with r = 0.999.



Performance Characteristics

Precision
Intra-assay coefficient of variation (CV) in the *Serazym*® Norovirus calculated from 8-fold determinations of samples:

sample	mean absorbance	standard deviation	CV (%)
1	2.132	0.043	2.00
2	0.902	0.035	3.93
3	0.534	0.017	3.28
4	0.217	0.013	5.92

Inter-assay coefficient of variation (CV) in the *Serazym*® Norovirus in 8 different test runs on 2 different days from 8-fold determinations of samples:

sample	mean absorbance	standard deviation	CV (%)
1	1.924	0.146	7.59
2	0.813	0.026	3.17
3	0.562	0.021	3.74
4	0.247	0.007	2.76

Lower detection limit
The lower detection limit of the *Serazym*® Norovirus was determined < 10 ng/ml capsid protein for genogroup I and II.

Specificity and sensitivity
One retrospective study with 159 stool specimens was performed to compare the *Serazym*® Norovirus with another commercially available ELISA.

	comparative ELISA positive	comparative ELISA negative
Serazym® ELISA positive	111	3
Serazym® ELISA negative	6	39

Specificity: 92.9% Sensitivity: 94.9%

Cross reactivity
Faecal samples positive for one of the following pathogens did not show any cross reaction in the *Serazym*® Norovirus:
Adenovirus (n = 6), *Astrovirus* (n = 8), *Rotavirus* (n = 6), *Clostridium difficile* (n = 8), *Campylobacter jejuni* (n = 6), *Helicobacter pylori* (n = 5), *Giardia lamblia* (n = 8), *Cryptosporidium parvum* (n = 7), *Entamoeba histolytica/dispar* (n = 6).

Negative stool specimens have been spiked with ≥ 10^8 colony forming units and virus particles per ml stool suspension resp. All microorganisms were tested negative with the *Serazym*® ELISA (OD 450 / 620 nm < Cut-Off):

Adenovirus	typ e41
Aeromonas hydrophila	ATCC 7966
Astrovirus	serotype 4
Bacillus cereus	ATCC 117788
Bacillus subtilis	ATCC 6633
Bacteroides fragilis	ATCC 25285
Campylobacter jejuni	ATCC 33291
Candida albicans	clinical isolate
Citrobacter freundii	ATCC 8090
Clostridium sordelli	ATCC 9714
Enterobacter aerogenes	ATCC 13048
Enterobacter cloacae	ATCC 13047
Enterococcus faecalis	ATCC 29212
Escherichia coli	ATCC 25922
Klebsiella pneumonia	ATCC 13883

Peptostreptococcus anaerobius	ATCC 27337
Proteus vulgaris	ATCC 8427
Pseudomonas aeroginosa	ATCC 10145
Rotavirus	strain SA11
Salmonella enterica Serovar thyphimurium	ATCC 14028
Salmonella enterica ssp. galolyticus	ATCC 13076
Shigella flexneri	ATCC 12022
Shigella sonnei	ATCC 25931
Staphylococus aureus	ATCC 25923
Staphylococcus epidermidis	ATCC 12228
Vibrio cholerae	RV 2011/ST5
Vibrio parahaemolyticus	ATCC 17802
Yersinia enterocolitica O:9	clinical isolate
Yersinia enterocolitica O:3	clinical isolate

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), Hemoglobine 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
2021-02-02	Common Advices and Precautions	Update
	History of Changes	Adding section “History of Changes“
	Test Components	Update
	Assay Procedure	Update

Incubation Scheme *Serazym*® Norovirus (E-061)

1.		pipette	
		100 µl	CONTROL + *(4)*
		100 µl	CONTROL – *(5)*
		100 µl	**diluted stool sample**
		60 min	incubation (room temperature)
		5 x wash	with wash solution
2.		3 drops (or 100 µl)	CONJ HRP *(6)*
		30 min	incubation (room temperature)
		5 x wash	with wash solution
3.		3 drops (or 100 µl)	SUBSTR TMB *(7)*
		10 min	incubation (room temperature) protected from light
4.		3 drops (or 100 µl)	STOP *(8)*

Read OD at 450 / ≥ 620 nm