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savyonDIAGNOSTICS

member of the gamida diagnostics division

savvy•gen GI- Astrovirus

REF: 609-01

Test kit for 48 determinations

Store at 2-37°C

For Professional Use Only IVD **CE**



Savyon® Diagnostics Ltd.

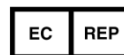
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Intended Use

The Savvygen™ GI- Astrovirus test allows the qualitative detection of Astrovirus by real time RT-PCR in human feces. The product is intended for use in the diagnosis of Astrovirus gastrointestinal infections alongside clinical data of the patient and other laboratory tests outcomes.

Background

Human Astroviruses (HAstV) are one of the most important causes of acute pediatric gastroenteritis. Classic Astroviruses can also be classified into eight genotypes, which correspond with eight serotypes. Type 1 is the most prevalent worldwide.

Classic HAstVs are distributed all over the world and are associated with 2 to 9% of cases of acute, nonbacterial diarrhea in children. HAstVs affect predominantly the pediatric population although infections in elderly people and immunocompromised hosts are also reported. Astroviruses are transmitted by the fecal-oral route, and outbreaks have been associated with consumption of sewage-polluted water and food. Typically, HAstV infection induces a mild, watery diarrhea that lasts 2 to 3 days, associated with vomiting, fever, anorexia, and abdominal pain.

The genome of human Astrovirus is composed of single-stranded, positive-sense RNA which encodes three open reading frames (ORFs), named ORF1a, ORF1b, and ORF2, being the ORF1b the best target for detection of Astrovirus.

Principles of the Procedure

The Savvygen™ GI- Astrovirus test is designed for the identification of Astrovirus in human feces specimens to aid in the assessment of infections caused by this virus.


The Savvygen™ GI- Astrovirus test is based on the real time amplification of specific conserved fragments of the genomic region ORF1b encoded by the Astrovirus genome. The viral RNA extracted is transcribed into cDNA using a specific primer by reverse transcription step followed immediately in the same well by polymerase chain reaction. The presence of Astrovirus is detected by an increase in observed fluorescence during the reaction upon hydrolysis of the fluorescent probe.

The assay is based on 5' nuclease chemistry which utilizes two primers and a hydrolysis fluorogenic probe to detect the accumulation of amplified target sequence during the PCR reaction. When the polymerase begins to extend the primers, the probe is hydrolyzed by its 5' to 3' exonuclease activity causing the spatial separation of reporter and quencher. The resulting increase in fluorescence signal is proportional to the amount of amplified product in the sample and detected by the real-time PCR instrument.

The Savvygen™ GI- Astrovirus test is a ready-to used test which contains in each well all the necessary reagents for real time PCR assay in a stabilized format. In addition, an internal control allows the detection of a possible reaction inhibition. The amplification of the target sequence is detected through the FAM channel whereas the internal control (IC) in HEX channel.



Materials/ Reagents Provided

Product Description	Contents
Savvygen™GI -Astrovirus 48 reactions. Cat.# 609-01 	6x Savvygen™GI- Astrovirus strips 1x Astrovirus Positive Control 1x Water RNase/DNase free 1mL 1x Rehydration Buffer 1.8 mL 1x Negative Control 1 mL Optical caps

Additional Equipment and Material Required

- Real Time PCR instrument (to check compatibility see Appendix I).
- RNA extraction kit.
- Centrifuge for 1.5 mL tubes.
- Vortex.
- Micropipettes (0.5-20 µL, 20-200 µL).
- Filter tips.
- Powder-free disposal gloves

Transport and Storage

The reagents and the test can be shipped at 2-37°C until expiration date stated in the label.

The re-suspended positive control should be stored at -20°C. In order to avoid repeated freeze/thaw cycles, we recommend separating into aliquots.

Keep all reagents in the dark.

Precautions

- This product is reserved exclusively for *in vitro* diagnostic purposes.
- Do not use after expiration date.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.
- Follow Good Laboratory Practices. Wear protective clothing, use disposal gloves, goggles and mask.
- Do not eat, drink or smoke in areas when samples or test reagents are being used. Once you finish the test wash your hands.
- Specimens must be treated as potentially infectious as well as all reagents and materials that have been exposed to the samples and handled in the same manner as an infectious agent. Take necessary precautions during the collection, storage, treatment and disposal of samples.
- Regular decontamination of commonly used equipment is recommended, especially micropipettes and work surfaces.

Test Procedure

Specimen Collection, Processing and RNA Extraction

Stool samples should be collected in clean containers and processed as soon as possible to guarantee the quality of the test. However, the samples can be frozen at -20°C for conservation. Ensure only the amount needed is thawed because of freezing and thawing cycles are not recommended.

For pretreatment and nucleic acid isolation, it is recommended to use your optimized manual or automatic system, and even any commercially available RNA extraction kit according to manufacturer's protocol. The assay has been validated with the following extraction kits:

- QIAamp MinElute Virus Spin Kit (QIAGEN).
- QIAamp Viral RNA Mini Kit (QIAGEN).
- Invisorb® Spin Universal Kit (Strattec).
- Ultraclean® Tissue & Cells RNA Isolation Kit (Mobio).
- NucleoSpin® RNA Virus (Machery Nagel).
- RIDA® Xtract (R-biopharm).
- Maxwell® RSC Blood DNA Kit, using the Maxwell® 16 instrument (Promega).

Positive Control Preparation

Reconstitute the lyophilized *Astrovirus* Positive Control (tube of red cap) with the 100 µL of Water RNase/DNase free (tube of white cap) supplied. To ensure a complete resuspension, vortex the tube thoroughly. After first use, dispense into aliquots in order to avoid multiple freeze-thaw cycles, and store them at -20°C.

This component contains high copies number template and is a very significant contamination risk. Therefore, we recommend open and manipulate it in a separate laboratory area away from the other components.

PCR Protocol

Thermo-cycler program

Calculate the number of required reactions including samples and controls (At least one positive and one negative control). Set your thermocycler following the conditions below:

Table 1. Real time RT-PCR conditions

Step	Temperature	Time	Cycles
Reverse transcription	45°C	15 min	1
Initial denaturation	95°C	2 min	1
Denaturation	95°C	10 sec.	45
Annealing/Extension (Data collection*)	60°C	50 sec.	

Set the fluorescence data collection during the extension step (*) through the FAM (*Astrovirus*) and HEX, JOE or VIC channels (Internal Control (IC)). If you use the Applied Biosystems 7500 Fast Real-Time PCR System, the Applied Biosystems StepOne™ Real-Time PCR System or the Stratagene Mx3005P™ Real Time PCR System check that passive reference option ROX is none.

a) Reconstitute the reaction mixture of the required wells.

Separate the number of required reactions including samples and controls. Remember that one positive and one negative control must be included in each run. Peel off protective aluminum seal from the strips/plate. Pipette 15 µL of Rehydration Buffer (tube of blue cap) into each well.



b) Adding samples and controls according to real-time PCR experimental plate set up.

Pipette 5 µL of Negative Control (tube of amber cap) into each negative control well. Pipette 5 µL of RNA sample into each sample well. Pipette 5 µL of reconstituted *Astrovirus* Positive Control (tube of red cap) into each positive control well. Cover the wells with the caps provided. Spin down briefly.

c) Performing PCR.

Place the strips/plate in the Real Time PCR instrument. Start the run.

Analysis and Interpretation of results

The analysis of the results is done by the software itself of the used real time PCR system following manufacturer’s instructions.

Positive control- The positive controls used in each run, must show an amplification curve for *Astrovirus*, which validates the reaction.

Negative control- The negative controls included in each run, must show the absence of signal for *Astrovirus*, which validates the reaction.

Internal control- The Internal Controls must show amplification curves, which verify the correct functioning of the amplification mix. Sometimes, the detection of internal control is not necessary because a high copy number of the pathogen RNA template can cause preferential amplification of target sequence.

Positive sample- A sample is assigned as positive for the target if the Ct value fall below 40. The internal control usually shows an amplification signal, although it might be dispensable if the amplification of the target sequence from a high copy number of RNA template can cause competition in the reaction.

Negative sample- A sample is assigned as negative for the target if there is no evidence of amplification signal in the detection system but the internal control is positive.

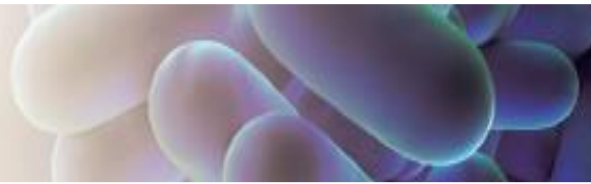
Invalid run- The assay should be considered as invalid and a new run should be performed if there is signal of amplification in negative control or absence of signal in the positive well.

Note: *If a negative sample do not show an amplification curve for the internal control, they should be retested by dilution of the original sample 1:10 or the nucleic acid extraction has to be repeated due to possible problems caused by PCR inhibitors*

Table 2: The result interpretation is summarized in the following table:

Astrovirus	Internal control	Negative control	Positive control	Interpretation
Positive	Positive/Negative	Negative	Positive	Astrovirus Positive
Negative	Positive	Negative	Positive	Astrovirus Negative
Positive	Positive	Positive	Positive	Experiment fail
Negative	Negative	Negative	Negative	Experiment fail

Positive: Amplification signal; **Negative:** No amplification signal



Limitations of the test

- This test provides a presumptive diagnosis of Astrovirus infection. All results must be interpreted together with other clinical information and laboratory findings available to the physician.
- This assay should be used only with samples from human feces. The use of other samples has not been established.
- The quality of the test depends on the quality of the sample; proper RNA from fecal specimens must be extracted. Unsuitable collection, storage and/or transport of specimens may give false negative results.
- Extremely low levels of target below the limit of detection may be detected, but results may not be reproducible.
- There is a possibility of false positive results due to cross-contamination by Astrovirus, either samples containing high concentrations of target RNA or contamination due to PCR products from previous reactions.

Quality Control

In order to confirm the appropriate performance of the molecular diagnostic technique, an Internal Control (IC) is included in each reaction. Besides, a positive and a negative control must be included in each assay to interpret the results correctly.

Performance Characteristics

Clinical sensitivity and specificity

Overall, 74 fecal samples from symptomatic patients were tested by Real Time PCR using: i) Savvygen™ GI- Astrovirus and ii) RIDA®GENE Viral Stool Panel II (r-Biopahrm). Astrovirus was detected in 40 samples by Savvygen™GI- Astrovirus test. This test identified even two additional low positives, but missed two Astrovirus positives detected by commercial kit. However, both samples has been evaluated by an additional commercial Real Time PCR Kit (Rotavirus/Norovirus/Astrovirus Real-TM (Sacace)), confirming our results.

The results show a high sensitivity and specificity to detect Astrovirus using Savvygen™GI- Astrovirus test.

Analytical sensitivity

This assay has a detection limit of ≥ 100 viral RNA copies per reaction (Figure 1).

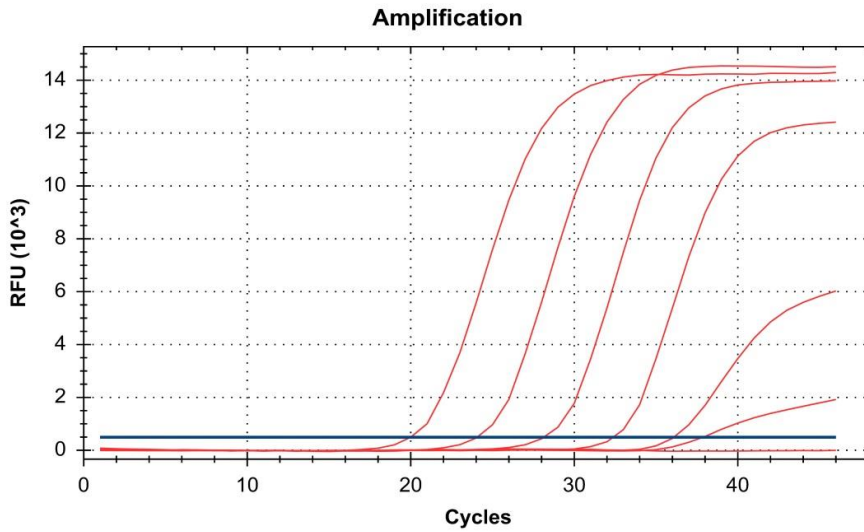


Figure 1. Amplification plot for 10-fold dilution series of Astrovirus template ranging from 10^7 to 10^2 copies/rxn.

Analytical specificity


The analytical specificity for Astrovirus was tested within the panel of following microorganisms, where no cross-reactivity was seen between any of the species.

Table 3. Cross-reactivity testing.

Adenovirus 40/41	<i>Cryptosporidium parvum</i>	<i>Salmonella paratyphi A</i>
Rotavirus A	<i>Entamoeba histolytica</i>	<i>Salmonella paratyphi B</i>
Norovirus GI and GII	<i>Enterococcus faecalis</i>	<i>Salmonella typhimurium</i>
<i>Aeromonas hydrophila</i>	Enterotoxigenic <i>E. coli</i> (ETEC)	<i>Salmonella bongori</i>
<i>Arcobacter butzleri</i>	Enteropathogenic <i>E. coli</i> (EPEC)	<i>Salmonella enteritidis</i>
<i>Bacteroides fragilis</i>	<i>Giardia intestinalis</i>	<i>Salmonella enterica</i>
<i>Campylobacter lari</i>	<i>Helicobacter pylori</i>	<i>Salmonella pullorum</i>
<i>Campylobacter fetus</i>	<i>Helicobacter hepaticus</i>	<i>Salmonella gallinarum</i>
<i>Campylobacter coli</i>	<i>Helicobacter cinaedi</i>	<i>Serratia liquefaciens</i>
<i>Campylobacter jejuni</i>	<i>Helicobacter heilmannii</i>	<i>Shigella flexneri</i>
<i>Campylobacter upsaliensis</i>	<i>Klebsiella oxytoca</i>	<i>Shigella dysenteriae</i>
<i>Candida albicans</i>	<i>Listeria monocytogenes</i>	<i>Staphylococcus aureus</i>
<i>Citrobacter freundii</i>	<i>Pseudomonas aeruginosa</i>	<i>Vibrio parahaemolyticus</i>
<i>Clostridium difficile</i>	<i>Proteus vulgaris</i>	<i>Y. enterocolitica</i> O:3 / O:9
<i>Clostridium perfringens</i>	<i>Salmonella typhi</i>	

Analytical reactivity

The reactivity of Savvygen™ GI- Astrovirus test was confirmed by the real time amplification using Astrovirus serotypes I-VIII as template.



Appendix A: Compatibility of the Savvygen GI Assays with Commercial Real-Time instruments

Savvygen™GI- Astrovirus assay has been validated on the following equipments: Applied Biosystems 7500 Fast Real-Time PCR System, Applied Biosystems StepOne™ Real-Time PCR System, Bio-Rad CFX96 Touch™ Real-Time PCR Detection System, AriaMx Real-Time PCR System, DNA-Technology DTPRime Real Time Detection Thermal Cycler. When using the Applied Biosystems 7500 Fast with strips it is recommend to place a plate holder to reduce the risk of crushed tube (Ref. PN 4388506). Additional compatible thermocyclers are listed below:

Applied Biosystems

- 7500 Fast Real-Time PCR System
- 7500 Fast Dx Real-Time PCR System
- QuantStudio™ 12K Flex 96-well Fast
- QuantStudio™ 6 Flex 96-well Fast
- QuantStudio™ 7 Flex 96-well Fast
- QuantStudio™ 3 Real-Time PCR System
- QuantStudio™ 5 Real-Time PCR System
- StepOne Plus™ Real-Time PCR System
- StepOne™ Real-Time PCR System
- ViiA™ 7 Fast Real-Time PCR System

Bio-Rad

- CFX96 Touch™ Real-Time PCR Detection System
- Mini Opticon™ Real-Time PCR Detection System

Roche

- LightCycler®480 Real-Time PCR System
- LightCycler®96 Real-Time PCR System

Agilent Technologies

- AriaMx Real-Time PCR System











DNA-Technology

- DTlite Real-Time PCR System
- DT prime Real-Time Detection Thermal Cycler

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1. Guix S, Bosch A, Pintó RM. Human astrovirus diagnosis and typing: current and future prospects. *Lett Appl Microbiol* 2005; 41(2): 103-105.
2. Morsy El-Senousy W, Guix S, Abid I, Pintó RM, Bosch A. Removal of astrovirus from water and sewage treatment plants, evaluated by a competitive reverse transcription-PCR. *Appl Environ Microbiol* 2007; 73(1): 164-167.
3. Wilhelmi I, Roman E, Sánchez-Fauquier A. Viruses causing gastroenteritis. *Clin Microbiol Infect* 2003; 9: 247-262.

Symbols for IVD Components and Reagents

 For <i>in vitro</i> diagnostic use only	 Keep dry	 Use by	 Manufacturer	 Lot number
 Consult instructions for use	 Temperature limitation	 Contains sufficient for <n> test	 DIL Buffer (sample diluent)	 Catalogue number

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