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- Diagnostik & molekulare Diagnostik
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savyonDIAGNOSTICS
member of the Gamida Diagnostics Division

savvy•gen GI- Adenovirus

REF: 607-01

Test kit for 48 determinations

Store at 2-37°C

For Professional Use Only IVD CE

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Intended Use

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

Background

Human adenoviruses (HAdV) are ubiquitous DNA viruses that cause a wide spectrum of illness. Adenoviruses are classified in the genus *Mastadenovirus*, which contains 7 known HAdV species (HAdV-A to HAdV-G). Historically, HAdVs have been classified by hemagglutination and serum neutralization reactions into 51 serotypes, although new adenovirus types identified by genomic data expand the types for a total of 57.

They are transmitted by the fecal-oral route and the respiratory route and are associated with acute respiratory disease (accounting for 10% of febrile respiratory diseases in children) and gastroenteritis. The enteric serotypes most frequently associated with the last symptom are 40 and 41, but additional serotypes 31, 12, 18, 1, 2, 5 and 6 have also been involved in the etiology of acute diarrhea.

Diagnosis of Adenovirus infections is currently based on virus isolation in cell culture or antigen detection. However, the need for rapid and sensitive detection methods has led to PCR being the best established among all other methods. The Real time PCR reaction targeting *hexon* gene is now a method of choice due to its sensibility and specificity

Principles of the Procedure

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

The Savvygen™ GI- Adenovirus test is based on the real time amplification of specific conserved fragments of the *hexon* gene encoded by the Adenovirus genome. After DNA extraction, Adenovirus 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- Adenovirus test is a ready-to use 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 -Adenovirus ***48 reactions*. Cat.# 607-01** 	6x Savvygen™GI- Adenovirus strips 1x Adenovirus 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).
- DNA 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 and stored at 2-37ºC until expiration date stated in the label.
The re-suspended positive control should be stored at -20ºC. 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 DNA 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 -20ºC for conservation. Ensure only the amount needed is thawed because of freezing and re-frosting 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 DNA extraction kit according to manufacturer´s protocol. The assay has been validated with the following extraction kits:

- *QIAamp DNA Mini kit (QIAGEN).*
- *QIAamp DNA Stool Mini Kit (QIAGEN).*
- *QIAamp MinElute Virus Spin Kit (QIAGEN).*
- *UltraClean® Tissue & Cells DNA Isolation Kit (Mobio).*
- *NucleoSpin® RNA Virus (Machery Nagel).*
- *RIDA ® Xtract (R-biopharm).*
- *Maxwell ® RSC Blood DNA Kit, using the Maxwell® 16 instrument (Promega).*
- *NucliSENS® EasyMAGTM platform (bioMérieux).*

Positive Control Preparation

Reconstitute the lyophilized *Adenovirus* Positive Control (tube of red cap) with 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 -20ºC.
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 PCR conditions

Step	Temperature	Time	Cycles
Polymerase activation	95ºC	2 min	1
Denaturalization	95ºC	10 sec.	45
Annealing/Extension (Data collection*)	60ºC	50 sec.	

Set the fluorescence data collection during the extension step (*) through the FAM (Adenovirus) 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 DNA sample into each sample well. Pipette 5 µL of reconstituted *Adenovirus* 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 Adenovirus, which validates the reaction.

Negative control- The negative controls included in each run, must show the absence of signal for Adenovirus, 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 DNA 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 DNA 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*

The result interpretation is summarized in the following table:

Table 2. Results interpretation

Adenovirus	Internal control	Negative control	Positive control	Interpretation
Positive	**Positive/Negative**	**Negative**	**Positive**	**Adenovirus Positive**
Negative	**Positive**	**Negative**	**Positive**	**Adenovirus 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 Adenovirus 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 DNA 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 Adenovirus, either samples containing high concentrations of target DNA 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, 95 faecal samples from symptomatic patients were tested by Real Time PCR using: i) Savvygen™ GI- Adenovirus; ii) RIDA®Gene Rotavirus/Adenovirus Duplex and iii) RIDA®GENE Viral Stool Panel II (r-Biopharm). Adenovirus was detected in 45 samples by Savvygen™ GI- Adenovirus. Among these, only 3 samples were not able to be detected by the other tests. The results show a high sensitivity and specificity to detect Adenovirus using Savvygen™ GI- Adenovirus.

Analytical sensitivity

This assay has a detection limit of ≥10 viral DNA copies per reaction (Figure 1).

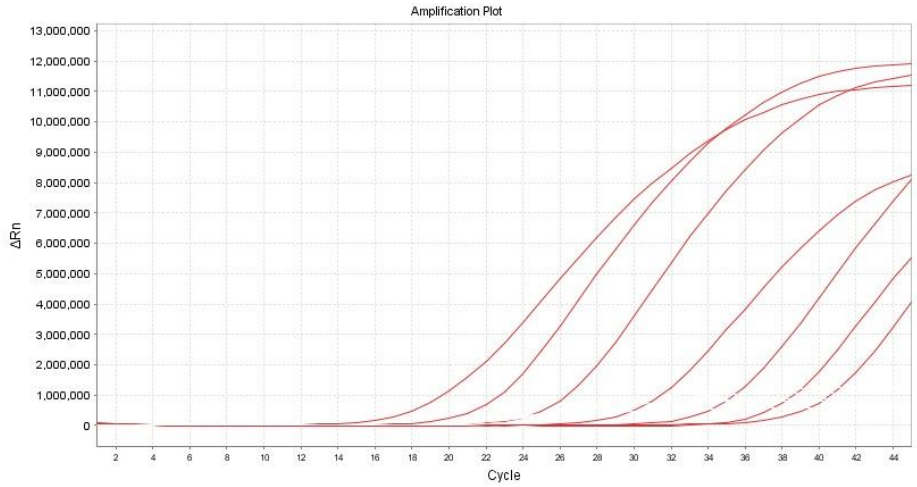

Figure 1. Amplification plot for 10-fold dilution series of Adenovirus template ranging from 10^7 to 10^1 copies/rxn

Analytical specificity

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

Table 3. Cross-reactivity testing

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

Analytical reactivity

The reactivity of Savvygen™ GI- Adenovirus test was confirmed by the real time amplification using Human Adenovirus 40, Human Adenovirus 41 strain Tak, Human Adenovirus type 1, Human Adenovirus 2 strain Adenoid 6, Human Adenovirus 5 and Human Adenovirus type 6 as template.

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

The Savvygen™ GI- Adenovirus test has been validated on the following equipment: 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. 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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3. Buckwalter SP, Teo R, Espy MJ, Sloan LM, Smith TF, Pritt BS. Real-time qualitative PCR for 57 human adenovirus types from multiple specimen sources. J Clin Microbiol 2012; 50(3): 766-771.
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Symbols for IVD Components and Reagents

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