savyonDIAGNOSTICS
Member of the Gamida Diagnostics Division

savvy•gen GI- Norovirus GI

REF: 610-01

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

Background

Noroviruses are a group of genetically diverse viruses that belong to the family *Caliciviridae* and that can be classified into 6 different genogroups, of which viruses from genogroup GI, GII and GIV are responsible for disease in humans. GI includes nine genotypes based on the complete major capsid protein (VP1), whereas to date, GII contains 22 genotypes. In humans, GII.4 viruses cause the majority of norovirus-related gastroenteritis outbreaks worldwide.

Noroviruses are transmitted primarily through the fecaloral route, either by direct person-to-person spread or fecally contaminated food or water. These viruses cause vomiting, nonbloody diarrhea, nausea, abdominal cramps and low-grade fever.

Noroviruses are positive-sense, single-stranded, nonenveloped RNA viruses. The linear RNA genome is organized in three open reading frames (ORFs). ORF1 encodes a large polyprotein, which is cleaved by the virus encoded protease into six non-structural proteins including the viral RNA polymerase. ORF2 and ORF3 encode the major and minor capsid proteins VP1 and VP2, respectively. The ORF1-ORF2 junction is the most highly conserved region of the genome and the best target for detection of Norovirus.

Principles of the Procedure

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

Savvygen™ GI- Norovirus GI test is based on the real time amplification of specific conserved fragments of the ORF1-ORF2 junction gene encoded by the Norovirus GI 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 Norovirus GI 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- Norovirus GI 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 -Norovirus GI ***48 reactions*. Cat.# 610-01** 	6x Savvygen™ GI- Norovirus GI strips 1x Norovirus 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 (to check compatibility see Appendix I).
- RNA extraction kit.
- Centrifuge for 1.5 mL tube.
- 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 to separate in aliquots.

Keep all reagents of 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 -20ºC 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 (Stratec).
UltraClean® Tissue & Cells RNA Isolation (Mobio).
NucleoSpin® RNA Virus (Machery Nagel).
RIDA® Xtract (R-biopharm).
Maxwell® RSC Blood DNA Kit, using Maxwell® 16 instrument (Promega).

Positive Control Preparation

Reconstitute the lyophilized *Norovirus GI* 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 -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 RT-PCR conditions

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

Set the fluorescence data collection during the extension step (*) through the FAM (Norovirus GI) 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 *Norovirus GI* 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 Norovirus GI which validates the reaction.

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

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The result interpretation is summarized in table 2:

Table 2. Results interpretation

Norovirus GI	Internal control	Negative control	Positive control	Interpretation
Positive	**Positive/Negative**	**Negative**	**Positive**	**Norovirus GI Positive**
Negative	**Positive**	**Negative**	**Positive**	**Norovirus GI 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 Norovirus GI 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 Norovirus GI, 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, 121 fecal samples from symptomatic patients were tested by Real Time PCR using: i) Savvygen™ GI- Norovirus GI and ii) RIDA®GENE Norovirus I&II (r-Biopharm). Norovirus GI was detected in 20 samples by Savvygen™ GI- Norovirus GI test. This test identified even one additional low positive that could be confirmed as positive by conventional RT-PCR and a low negative that could be confirmed as negative by a conventional RT-PCR as well.

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

Analytical sensitivity

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

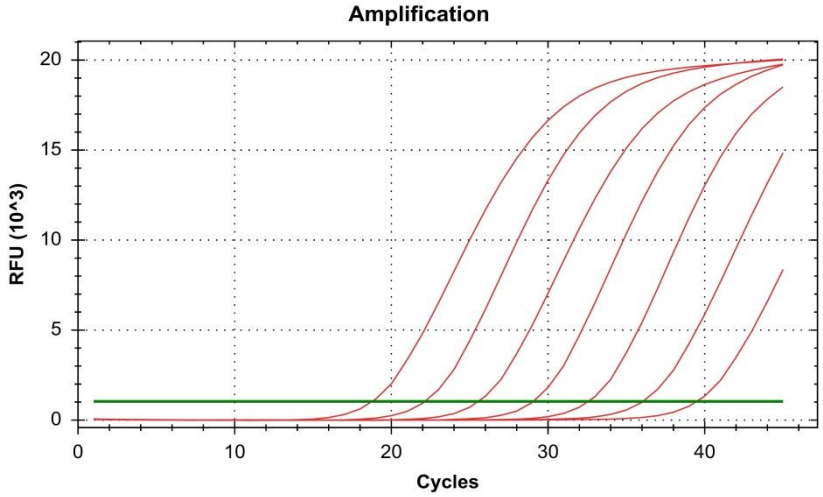

Figure 1. Amplification plot for 10-fold dilution series of Norovirus GI template ranging from 10^7 to 10^1 copies/rxn.

Analytical specificity

The analytical specificity for Norovirus GI 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	*Cryptosporidium parvum*	*Salmonella paratyphi A*
Astrovirus Genotype I-VIII	*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 the Savvygen™ GI- Norovirus GI test was confirmed by the real time amplification using Norovirus GI genotypes GI.1, GI.2, GI.3, GI.4, GI.5, GI.7, GI.8, GI.9, GI.P2, GI.P3, GI.P4, GI.P7, GI.P9 and GI.Pb as template.

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

Savvygen™GI- Norovirus GI 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 TouchTM 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

Bibliography

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2. Trujillo AA, McCaustland KA, Zheng DP, Hadley LA, Vaughn G, Adams SM, Ando T, Glass RI, Monroe SS. Use of TaqMan real-time reverse transcription-PCR for rapid detection, quantification, and typing of norovirus. J Clin Microbiol 2006; 44(4): 1405-1412.
3. Vinjé J. Advances in laboratory methods for detection and typing of norovirus. J Clin Microbiol 2015; 53(2): 373-381.
4. Hoa Tran TN, Trainor E, Nakagomi T, Cunliffe NA, Nakagomi O. Molecular epidemiology of noroviruses associated with acute sporadic gastroenteritis in children: global distribution of genogroups, genotypes and GII.4 variants. J Clin Virol 2013; 56(3): 185-193.

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

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