



savyonDIAGNOSTICS

member of the gamida diagnostics division

savvy•genGI- Salmonella

REF: 604-01

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

For *in-vitro* professional diagnostic use.

Background

Salmonella is a genus of bacteria consists of a gram-negative rod-shaped bacillus, facultative anaerobic, usually motile and flagellated, which has being reported as one of the pathogenic agents with major impact on public health.

The genus Salmonella contains two distinct species, designated *Salmonella enterica* (divided into six subspecies) and *Salmonella bongori*. Salmonella species are the major causative agents of acute bacterial gastroenteritis. Although the impact on global human health caused by Salmonella has not been estimated, gastroenteritis is a major cause of morbidity and mortality in children under 5 years old and in the general population.

The main source of transmission of human salmonellosis is contaminated food with non-typhoidal Salmonella, being the most common poultry and poultry products, followed by others such as beef, pork, eggs, milk and seafood. Other sources of transmission could be contaminated water or contact with infected animals. Clinical manifestations range from asymptomatic colonization to systemic life-threatening infections.

There are different methods for the specific detection of Salmonella, including Real time PCR targeting the *invA* gene.


Principles of the Procedure

Savvygen™GI- Salmonella test is designed for the identification of Salmonella in human feces specimens to aid in the assessment of infections caused by these bacteria. The test is based on the real time amplification of specific conserved fragments of the *invA* gene encoded by the Salmonella genome. After DNA extraction, Salmonella 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.

Savvygen™GI- Salmonella 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 Salmonella; 48 reactions. Cat.# 604-01 	6x Savvygen™GI- Salmonella strips 1x Salmonella 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 2-37°C until the expiration date stated on the label.
- Positive control after resuspension should be kept at -20°C. In order to avoid repeated freeze/thaw cycles, it is recommend separating it 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.
- Separate pre-amplification steps from post-amplification steps. Use separate locations for pre- and post-amplification. Use dedicated lab equipment for each stage. Prepare samples in a laminar flow hood using dedicated equipment to minimize contamination. Set up the post-amplification area in a low-traffic area with dedicated equipment. Follow Good Laboratory Practices. Wear protective clothing, use disposal gloves, goggles and mask.
- Use disposable containers, disposable barrier pipette tips, disposable bench pads, and disposable gloves. Avoid washable lab wear.
- Use a diluted bleach solution (0.2% sodium hypochlorite) to treat waste from the post-amplification and detection areas, as the waste contains amplicon. Use the bleach solution to wipe down equipment and bench areas, and to treat drains used to dispose of liquid waste.
- Do not eat, drink or smoke in areas when samples or test reagents are being used
- 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. The samples can be frozen at -20°C for longer time periods. Ensure only the amount needed is thawed because of freezing and refrosting 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:

- *Invisorb® Spin Universal Kit (Strattec).*
- *QIAamp DNA Stool Mini Kit (QIAGEN).*
- *QIAamp DNA Mini kit (QIAGEN).*
- *Maxwell® RSC Blood DNA Kit, using the Maxwell® 16 instrument (Promega).*
- *RIDA ® Xtract (r-Biopharm)*
- *UltraClean® Tissue & Cells DNA Isolation Kit (Mobio)*

Positive Control Preparation

Reconstitute the lyophilized Salmonella Positive Control (red cap tube) with 100 µL of Water RNase/DNase free (white cap tube) supplied, vortex the tube thoroughly. After first use, dispense the Positive Control into aliquots in order to avoid multiple freeze-thaw cycles, and store 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 thermos-cycler following the conditions below (Table 1):

Table 1. Real time PCR conditions

Step	Temperature	Time	Cycles
Polymerase activation	95°C	2 min	1
Denaturalization	95°C	10 sec.	45
Annealing/Extension	60°C	50 sec.	

Set the fluorescence data collection during the extension step (*) through **the FAM (Salmonella) 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.

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

b) Adding samples and controls according to real-time PCR experimental strips/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 Salmonella Positive Control (tube of red cap) into each positive control well. Cover the wells with the caps provided. Spin down briefly.

c) PCR Run.

Place the strips in the Real-Time PCR instrument and 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 control used in each run must show an amplification curve for Salmonella, which validates the reaction.

Negative control- The negative control included in each run must show the absence of signal for Salmonella, 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 falls 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*

Table 2. Results interpretation

<i>Salmonella</i>	Internal control	Negative control	Positive control	Interpretation
Positive	Positive/Negative	Negative	Positive	<i>Salmonella</i> Positive
Negative	Positive	Negative	Positive	<i>Salmonella</i> 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 Salmonella 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 Salmonella, 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, 74 fecal samples from symptomatic patients were tested by Real Time PCR using: i) Savvygen™GI- Salmonella test; and ii) RIDA®GENE Bacterial Stool Panel (R-Biopharm). Salmonella was detected in 31 samples by Savvygen™GI- Salmonella test. The results show a high sensitivity and specificity to detect Salmonella using Savvygen™GI- Salmonella test.

Analytical sensitivity

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

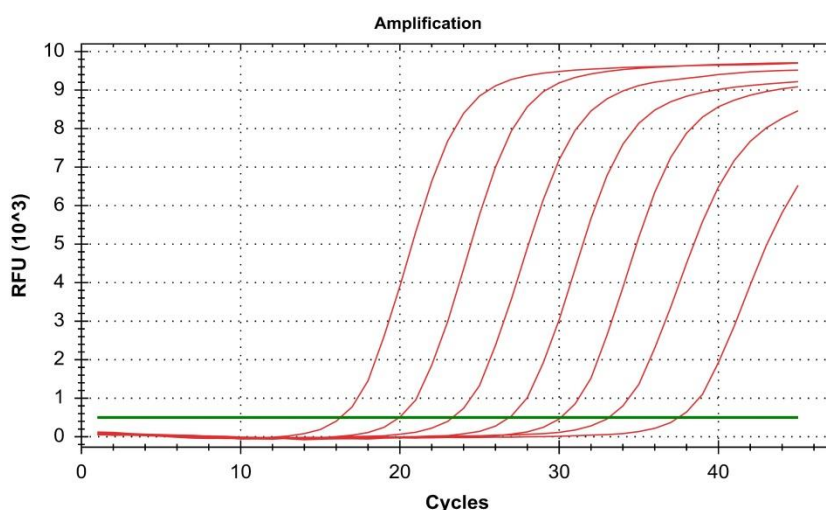


Figure 1. Amplification plot for 10-fold dilution series of *Giardia lamblia* template ranging from 10^7 to 10^1 copies/reaction.

Analytical specificity

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

Table 3. Cross-reactivity testing.

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

Analytical reactivity

Savvygen™ GI- Salmonella test was confirmed by the real time amplification using *Salmonella enterica* subsp. *enterica*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Salmonella typhi*, *Salmonella bongori*, *Salmonella pullorum* and *Salmonella gallinarum*, *Salmonella mbandaka* and *Salmonella braenderup* as template.



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

Savvygen™ GI- Salmonella 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 thermos-cyclers 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

1. Rodriguez-Lázaro D, Hernández M, Esteve T, Hoorfar J, Pla M, Majowicz SE. A rapid and direct real time PCR-based method for identification of *Salmonella* spp. J Microbiol Methods 2003; 54(3): 381-390.
2. Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM; International Collaboration on Enteric Disease 'Burden of Illness' Studies. The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin Infect Dis 2010; 50(6): 882-889.
3. Barletta F, Mercado EH, Lluque A, Ruiz J, Cleary TG, Ochoa TJ. Multiplex real-time PCR for detection of *Campylobacter*, *Salmonella*, and *Shigella*. J Clin Microbiol 2013; 51(9): 2822-2829.

Symbols for IVD Components and Reagents

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

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