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- Diagnostik & molekulare Diagnostik
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siehe unsere Liefer- und Versandbedingungen

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savyonDIAGNOSTICS
member of the Gamida Diagnostics Division

savvy•gen GI- Entamoeba

REF: 603-01
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- Entamoeba test is intended for qualitative detection of *Entamoeba histolytica* by real time PCR in human feces. The test is intended to be used in the clinical laboratories for diagnosis of *Entamoeba histolytica* gastrointestinal infections alongside clinical data of the patient and other laboratory tests outcomes.

For *in-vitro* professional diagnostic use.

Background

There are many species in the genus Entamoeba, of which, *E. histolytica, E. dispar, E. moshkovskii, E. coli, E. hartmanni,* and *E. polecki* are found in the intestinal lumen of humans. *E. histolytica* is a parasitic organism responsible for significant morbidity and mortality, mainly in developing countries and several communities of developed nations. Manifestations of amebiasis range from asymptomatic colonization to dysentery and extra intestinal invasive disease, most commonly in the form of liver abscesses. The disease is spread primarily by food or water contaminated with cysts but may also be transmitted from person to person. It is highly prevalent in regions of the world where personal hygiene and/or sanitation are insufficient.

Entamoeba infections are traditionally diagnosed via microscopic examination of stool samples, fresh or fixed. However, it has been shown that the sensitivity and specificity of microscopy in differentiating the Entamoeba species is less optimal than other diagnostic methods. In fact, the pathogenic amoeba, *E. histolytica* is indistinguishable in its cyst and trophozite stages from E. dispar and E. moshkovskii. Given the discrepancies of microscopy, the epidemiology of Entamoeba can be further studied by PCR assay targeting the 18S rRNA genes.

Principles of the Procedure

Savvygen™GI- Entamoeba test is designed for the identification of *Entamoeba histolytica* in human feces specimens to aid in the assessment of infections caused by this parasite. The test is based on the real time amplification of specific conserved fragments of the 18S rRNA gene encoded by the *Entamoeba histolytica* genome. After DNA extraction, *Entamoeba histolytica* 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- Entamoeba 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 Entamoeba histolytica ***48 reactions*****. Cat.# 603-01** 	6x Savvygen™GI- Entamoeba strips 1x Entamoeba histolytica 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 the expiration date stated on the label.
- Positive control after resuspension should be kept at -20ºC. 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 -20ºC 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 (Stratec).*
- *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 Entamoeba histolytica 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 -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 thermos-cycler following the conditions below (Table 1):

Table 1. Real time PCR conditions

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

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

Negative control- The negative control included in each run must show the absence of signal for Entamoeba histolytica, 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

Entamoeba histolytica	Internal control	Negative control	Positive control	Interpretation
Positive	**Positive/Negative**	**Negative**	**Positive**	***Entamoeba histolytica* Positive**
Negative	**Positive**	**Negative**	**Positive**	***Entamoeba histolytica* 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 Entamoeba histolytica 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 Entamoeba histolytica, 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, 149 faecal samples from symptomatic patients were tested by Real Time PCR using: i) Savvygen™GI- Entamoeba test; and ii) RIDA®GENE Parasitic Stool Panel II (R-Biopharm). Entamoeba histolytica was detected in 2 spiked samples by Savvygen™GI- Entamoeba test.. The results show a high sensitivity and specificity to detect Entamoeba histolytica using Savvygen™GI- Entamoeba test.

Analytical sensitivity

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

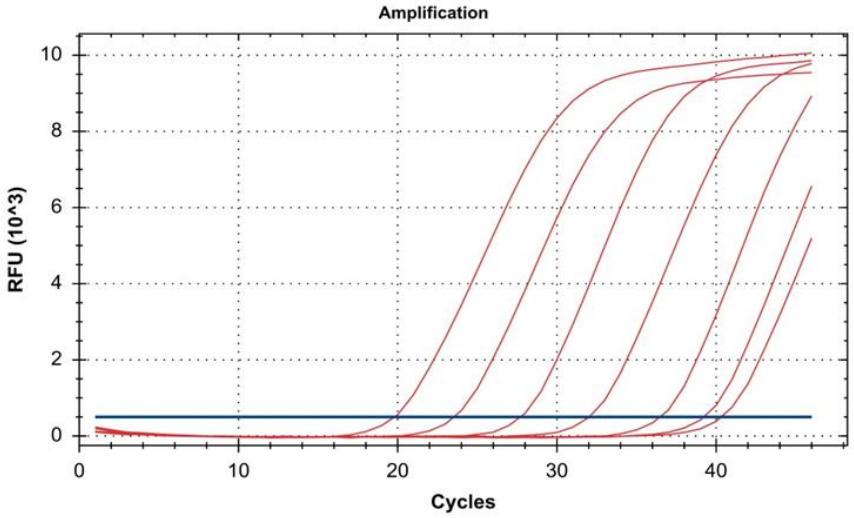

Figure 1. Amplification plot for 10-fold dilution series of Entamoeba histolytica template ranging from 10^7 to 10^1 copies/reaction.

Analytical specificity

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

Analytical reactivity

The reactivity of Savvygen™GI- Entamoeba test.was confirmed by the real time amplification using Entamoeba histolytica strain DS4-868 as template.

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

Savvygen™GI- Entamoeba 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 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

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3. Lau YL, Anthony C, Fakhrurrazi SA, Ibrahim J, Ithoi I, Mahmud R. Real-time PCR assay in differentiating *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* infections in Orang Asli settlements in Malaysia. Parasit Vectors 2013; 6(1): 250.

Symbols for IVD Components and Reagents

IVD	For *in 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