savyonDIAGNOSTICS
member of the Gamida Diagnostics Division

savvy•gen STI MG/MH

REF **615-01**

Test kit for 48 determinations


≤-20°C

For Professional Use Only

CE IVD

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Intended Use

The Savvygen™ STI MG/MH allows the qualitative detection of *Mycoplasma genitalium* (MG) and *Mycoplasma hominis* (MH) by Real- time PCR in urine or from vaginal swab specimen. The test is intended to be used as an aid in the diagnosis of *Mycoplasma genitalium* and *Mycoplasma hominis* infections, in both symptomatic and asymptomatic individuals.

For *in-vitro* professional diagnostic use.

Background

According to the World Health Organization (WHO) more than 340 million new cases of sexually transmitted bacterial and protozoan infections occur throughout the world every year (1). Sexually transmitted diseases (STDs) also known as Sexually Transmitted Infections (STIs) consist of diseases that are spread primarily through person-to-person sexual contact. STIs represent a significant public health concern. Although many STIs remain asymptomatic or do not exhibit clear and distinctive symptoms, such infections may result in acute symptoms as well as other severe delayed consequences, such as infertility, ectopic pregnancy, cervical cancer and death (2; 3). These diseases are caused by more than 30 pathogenic viruses, bacteria and parasites (1). Furthermore, infection by STDs increases substantially the potential of acquiring or transmitting human immunodeficiency virus HIV. Such interaction could account for 40% or more of HIV transmissions (1; 3). The Savvygen™ STI MG/MH was developed in order to detect two of the most common sexually transmitted pathogens- *Mycoplasma genitalium* (MG) and *Mycoplasma hominis (*MH).

Mycoplasma genitalium - is a small parasitic bacterium that attacks ciliated epithelial cells of the urinary and genital tracts in humans. Its existence was first reported in 1981 and was eventually identified as new species of *Mycoplasma* in 1983. It is a sexually transmitted pathogen which can cause significant morbidity in men and women, and is a co-factor in HIV transmission. A *Mycoplasma genitalium* infection is quite similar to that of *C. trachomatis* and *N. gonorrhoea*, but often occurs together with other STIs, making it difficult to diagnose. It infects both men and women, it is often associated with bacterial vaginosis (BV), and is a common cause of non-gonococcal urethritis in men. Such an infection can remain unrecognized for months when it is asymptomatic. It can cause prostatitis or epididymitis in men and cervicitis, urethritis, or ectopic pregnancy in women. If untreated, *M. genitalium* can also leave both men and women infertile and lead to long-lasting pelvic pain (4, 5, 6).

Mycoplasma hominis - Along with *M. pneumoniae, M. genitalium* and *Ureaplasma urealyticum, Mycoplasma hominis* is one of the four mycoplasma species for which the pathogenic potential in humans is certain. It is an opportunistic bacterium which has the highest probability of being the cause of genital infections in women. *M. hominis* may also cause infections of the newborn and extra genital infections, especially in immunosuppressed subjects. It is also believed to be a cause of pelvic inflammatory disease, as one of other possible known causes. In addition, this bacterium has also been associated with postabortal and postpartum fever (7, 8, 9).

Principles of the Test

The Savvygen™ STI MG/MH is a ready-to use test which contains all the necessary reagents for real time PCR assay. The test is designed for the diagnosis of *Mycoplasma genitalium* and/or *Mycoplasma hominis* in human urine specimens or vaginal swabs to aid in the assessment of infections caused by these sexually transmitted bacteria.

The Savvygen™ MG/MH test is based on amplification of highly specific conserved fragments in the gyrA gene of *Mycoplasma genitalium* and *gap* gene of *Mycoplasma hominis.* Following extraction of bacterial DNA the fragments are amplified by Taq DNA in Polymerase Chain Reaction (PCR). The assay is based on the 5'→3' exonuclease activity of Taq DNA Polymerase (figure 1). A fluorophore/quencher dual-labeled probe is annealing to an internal specific sequence. Upon primer elongation, Taq DNA Polymerase displaces and hydrolyzes the probe, thus releasing and activating the fluorophore. The presence of the bacteria is detected by an increase in

the observed fluorescence during the reaction. The resulting increase in fluorescence signal is proportional to the amount of amplified product in the sample and detected by the real-time PCR thermocycler.

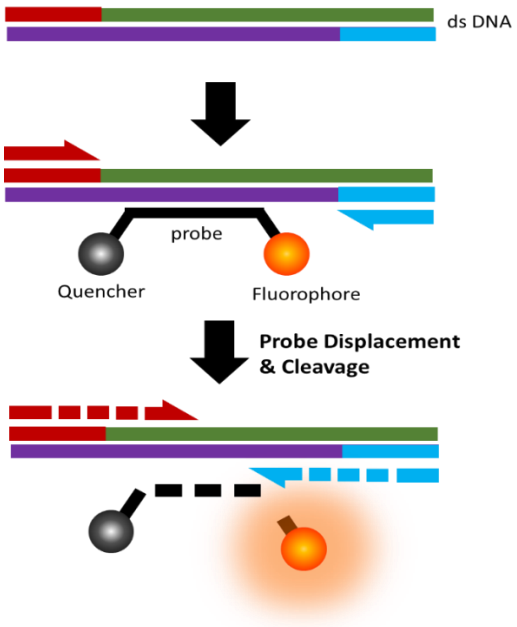

Figure 1. *Principle of the Savvygen™ assay*

The optical channels used for multiplexed detection of the amplified fragments are outlined in table 1 below:

Table 1. Savvygen™ STI MG/MH target and optical channel detection;

Name	**LightCycler ®96**	**Bio-Rad CFX96**		**ABI 7500 Real-Time PCR**
MG	FAM	FAM		FAM
MH	HEX	HEX		VIC
Internal Control	Red610	Cal Red610		ROX

*In ABI 7500 Real-Time PCR, ROX channel as a reference dye is un-marked

Materials/ Reagents Provided

Product Description	Contents
Savvygen™ STI MG/MH *48 reactions. Cat.# 615-01*	3x Savvygen MG/MH Master Mix* vials (264 µL each)
	1x Savvygen MG/MH positive Control (50 µL each)

* Master-mix vial contains all reagents required for the amplification reaction

Additional Equipment and Material Required

- **Reagents**
  - DNA extraction kit.
  - Ultrapure water (for negative control)
- **Disposables**
  - Micropipettes (0.5-20 µL, 20-200 µL).
  - Powder-free disposal gloves
  - PCR instrument plate or strips
- **Instruments**
  - Centrifuge for 1.5 mL tube.
  - Real-Time PCR instrument (see table 2A+2B for compatible RT-PCRs).

Note: *Savvygen™ STI* MG/MH *has been validated on the: Bio-Rad CFX96 Touch™ Real-Time PCR Detection System, Roche LightCycler®96 Real-Time PCR System and 7500 Real-Time PCR System*

Table 2A. Compatible Real Time PCR instruments (Low-Profile)

Bio-Rad	***Applied Biosystems***
CFX96 Touch™ Real-Time PCR Detection System	7500 Fast Real-Time PCR System
Roche	7500 Fast Dx Real-Time PCR System
LightCycler ®480 Real-Time PCR System	QuantStudio™ 12K Flex 96-well Fast
LightCycler ®96 Real-Time PCR System	QuantStudio™ 6 Flex 96-well Fast
Agilent Technologies	QuantStudio™ 7 Flex 96-well Fast
AriaMx Real-Time PCR System	QuantStudio™ 5 Real-Time PCR System
DNA-Technology	ViiA™ 7 Fast Real-Time PCR System
DTlite Real-Time PCR System	***Cepheid***
DT prime Real-Time Detection Thermal Cycler	SmartCycler®*
	Qiagen
	*Rotor-Gen® Q**

Table 2B. Compatible Real Time PCR instrument (High-Profile)

Bio-Rad	***Applied Biosystems***
CFX96 Touch Deep Well Real-Time PCR Detection System	*7500 Real-Time PCR System*
iCycler iQ Real-Time PCR Detection System	*QuantStudio™ 12K Flex 96-well*
iCycler iQ 5 Real-Time PCR Detection System	*QuantStudio™ 6 Flex 96-well*
DNA-Technology	*QuantStudio™ 7 Flex 96-well*
DTlite Real-Time PCR System	*QuantStudio™ 5 Real-Time PCR System*
DT prime Real-Time Detection Thermal Cycler	*ViiA™ 7 Real-Time PCR System*
Stratagene /Agilent Technologies	***Qiagen***
Mx3000P™ Real Time PCR System	*Rotor-Gen® Q**
Mx3005P™ Real Time PCR System	***Cepheid***
Analytik Jena Biometra	*SmartCycler®**
TOptical	***Abbot***
qTOWER 2.0	*Abbot m2000 RealTime System*

Transport and Kit Storage

The Savvygen™ MG/MH *kit* should be transported and stored at -20ºC. All components are stable under recommended storage conditions until the expiry date as stated on the label.
Note: Each Master-mix vial is good for up to 3 freeze/thaw cycles. After 3 uses the vial should be disposed according to Good Laboratory Practice guidelines..

Precautions

Amplification technologies can amplify target nucleic acid sequences over a billion-fold, providing an efficient way for detecting very low target concentrations. Care must be taken to avoid contamination of samples with target nucleic acids from other samples, or amplicons from previous amplifications. The following are recommendations to help contamination control:

1. Separate pre-amplification from post-amplification steps. Use separate locations for pre- and post-amplifications. Use dedicated lab equipment for each stage. Prepare samples in a laminar flow hood using dedicated equipment. Set up the post-amplification area in a low-traffic area as possible.
2. The laboratory process must be one-directional. It should start 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.
3. Use disposable containers, barrier pipette tips, bench pads, and gloves. Avoid washable lab wear.
4. Use a diluted bleach solution (0.2% sodium hypochlorite) to treat waste from the post-amplification and detection areas, as the waste contains amplicons. Use the bleach solution to wipe down equipment and bench areas, as well as to treat drains used for disposal of liquid waste.
5. Use negative controls to detect possible contaminations during the reaction setup. If reagent contamination is detected, dispose the suspected reagents.
6. Do not use reagents after the expiration date stated on the box.
7. Specimens must be treated as potential infectives as well as all reagents and materials that had been exposed to the samples. All these should be handled in the same manner as an infectious agent. Take necessary precautions during the collection, storage, treatment and disposal of samples.

Test Procedure

Specimen Collection, Processing and DNA Extraction

In order to obtain an adequate sample, the procedure for sample collection must be followed closely and according to the manufacturer's instructions. The specimens should be transported as fast as possible and stored at the indicated temperature conditions.

Nucleic Acid (NA) Extraction: for pre-treatment and NA isolation of the specimens, it is recommended to use an appropriate DNA extraction kit according to manufacturer´s protocol. NA Extraction may be carried out manually or automatically using commercially available extraction kits. Several extraction systems were validated for this kit including:

- Savyon STI Extraction kit (Savyon Diagnostics Cat#651-01; 652-01)
- QIAamp DNA mini kit (Qiagen)
- Nimbus IVD (Hamilton)
- NucliSENS® easyMag® (Biomérieux)

Programming the Real-Time PCR Instrument

Thermal profile

Set your thermocycler to the following conditions below (table 3):

Table 3. PCR program profile for the Savvygen™ MG/MH

Step	Temperature (ºC)	Time	Cycles
Polymerase activation	95	1 min	1
Denaturation Annealing/Extension	95 60 *****	5 sec 30 sec	45

***** *Set the fluorescence data collection during the extension step (*) through the FAM, Red610, Cy5, and HEX, JOE or VIC channels.*

Note: Set the reaction volume to **20µl**.

Fluorescence reading

Select the detection channels on your Real-Time PCR instrument as appear below:

Name	LightCycler ®96	Bio-Rad CFX96	ABI 7500 Real-Time PCR
MG	FAM	FAM	FAM
MH	HEX	HEX	VIC
Internal Control	Red610	Cal Red610	ROX

*In ABI 7500 Real-Time PCR, ROX channel as a reference dye is un-marked

Preparing reaction wells

Calculate the number of required reactions including samples and controls. It is highly recommended to run at least one positive and one negative control per run.

1. Thaw the Master mix / Positive Control tube.
2. Before use, mix by inverting the tube 2-3 times and then do a short spin down. **Do not vortex!**

***Adding reagents,* samples and controls into the reaction plate / strip**

3. Pipette 15 µL of the Master mix into each well.
4. Pipette 5 µL of DNA sample into each sample well.
5. Pipette 5 µL of the Positive Control into the positive control well.
6. Pipette 5 µL of Negative Control (Ultra-pure Water; not provided) into each negative control well.
7. Cover the wells with the suitable caps/seal. Spin down briefly if needed.

Performing PCR

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

The fluorescence detection channels of common Real Time PCR Thermocyclers are specified in Table 4.

Table 4: Detection fluorescence channels of different Real Time PCR systems

RT- PCR THERMOCYCLER	*System Detection channels*	*Savvygen probes channels*	*Remarks*
Roche LightCycler® 96 or LightCycler®480II	465/510	FAM	Color Compensation is required only for LC480 system
	533/580	HEX	
	533/610	ROX	
	618/660	Cy5	
Applied Biosystems ABI 7500 fast	FAM	FAM	Passive reference option ROX is not mark
	VIC	HEX	
	ROX	ROX	
	Cy5	Cy5	
Bio-Rad CFX96 ™	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
	Cy5	Cy5	
Agilent AriaMx	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
	Cy5	Cy5	
DNA-Technology DTlite / DTprime	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
	Cy5	Cy5	
Smartcycler® Cepheid	Channel 1	FAM	
	Channel 2	HEX	
	Channel 3	ROX	
	Channel 4	Cy5	
Abbott m2000rt	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
	Cy5	Cy5	
Rotor-Gene®Q Qiagen	Green	FAM	
	Yellow	HEX	
	Orange	ROX	
	Red	Cy5	

Analysis of Results

Interpretation of results (table 5) can be automatically performed if programed by the user using the RT-PCR instrument software following manufacturer´s instructions. It is required to run assay controls (positive and negative controls) in each run to validate the reaction.

Note: *The positive controls used in each run, must show an amplification curve of the tested targets which validates the reaction while the negative control well should demonstrate an absence of signal (except internal control target).*

Positive sample- A sample is considered a positive for the target in the presence of amplification curve signal.

Negative sample- A sample is considered negative for the target if there is no evidence of amplification curve signal in the detection system and the internal control is positive.

Internal control- The Internal Control must show an amplification curve, which verifies the correct function of the amplification mix. It should be noted that the detection of internal control may not be necessary in case of positive results in one or more of the pathogens.

Positive control- The positive control used in each run is expected to show an amplification curve for the 2 bacteria which validates the reaction.

Negative control- The negative controls included in each run is expected to show no curve amplification signal for the 2 STI bacteria which validates the reaction.

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

Note: If an amplification curve for the internal control is not shown, it may be due to PCR inhibitors. In such case the sample should be retested by dilution of the original sample 1:10. Alternatively it is recommended to repeat the nucleic acid extraction.

Table 5. Interpretation of results.

M. genitalium (FAM)	*M. hominis* (HEX)	Internal control (Red610)	Negative control	Positive control	**Interpretation**
POS	POS	POS / NEG	NEG	POS	*M. genitalium* , *M. hominis* **Positive**
NEG	NEG	POS	NEG	POS	*M. genitalium* , *M. hominis* **Negative**
POS	NEG	POS / NEG	NEG	POS	*M. genitalium* , **Positive**, *M. hominis* **Negative**
NEG	POS	POS / NEG	NEG	POS	*M. genitalium* , **Negative**, *M. hominis* **Positive**
POS	POS	POS	POS	POS	**Invalid run**
NEG	NEG	NEG	NEG	NEG	**Invalid run**
NEG/POS	NEG/POS	POS	NEG	NEG	**Invalid run**

POS: presence of amplification curve signal
NEG: No amplification curve signal

Limitations of the Test

- All results should be used and interpreted in the context of a full clinical evaluation as an aid in the diagnosis of sexually transmitted infections.
- This test was only validated for urine and/or vaginal swabs.
- Error results may occur due to improper sample collection, handling, storage, technical error, sample mix-up, or the amount of organisms in the sample which is below the analytical sensitivity of the test.
- The presence of PCR inhibitors may cause invalid results.
- A false positive result with other targets may derive from contamination by PCR products from previous testing.
- As with all PCR-based *in-vitro* diagnostic tests, extremely low levels of target below the analytical sensitivity of the assay may be detected, but results may not be reproducible.
- If a certain sample result is Invalid then the sample should be repeated starting from DNA extraction stage.

Quality Control

In order to confirm the appropriate performance of the molecular diagnostic technique, an Internal Control (IC) is included in each reaction. In addition, positive and negative controls are included in each assay to enable correct interpretation of the results.

Performance Characteristics

Clinical sensitivity and specificity

Clinical performance characteristics of the Savvygen™ STI MG/MH were assessed by evaluation of retrospective (frozen) specimens (DNA, urine and swabs). These specimens are consisted of residual anonymized urine and swabs collected and processed in various clinical laboratories, which were verified by CE approved molecular methods. The performance of the Savvygen™ STI MG/MH test is presented in table 6:

Table 6. Results interpretation

Pathogen	Positive Agreement		Specificity	
	TP/ (TP+FN)	Percent	TN/ (TN+FP)	Percent
Mycoplasma genitalium	86/86	100%	155/155	100%
Mycoplasma hominis	118/118	100%	123/123	100%

TP- True Positive; TN- True Negative; FP- False Positive; FN- False Negative

Analytical sensitivity and specificity

Analytical sensitivity

Serial dilutions of samples containing a plasmid with the targets sequences were tested in three different batches in consecutive experiments. Six replicates of each dilution were tested per target (pathogen) and the limit of detection (LOD) was set accordingly to the last dilution in which all replicates were identified. LOD for all the pathogens in the assay is presented in Table 7:

Table 7: LOD (copy/reaction) of the Savvygen™ MG/MH

Pathogen	Copy No./Reaction
Mycoplasma genitalium	30
Mycoplasma hominis	38

Analytical specificity

In order to detect possible cross-reactions of the Savvygen™ STI MG/MH, samples positive for potential cross-reactive pathogens were tested. None of the tested pathogens revealed a positive result.

Table 8: Cross-Reactivity experiment

	Mycoplasma genitalium (FAM)	*Mycoplasma hominis (HEX)*
Neisseria lactamica	-	-
Dientamoeba fragilis	-	-
Campylobacter jejuni	-	-
candida albicans	-	-
Yersinia type 4	-	-
Yersinia type 2 (Culture)	-	-
Human papillomavirus type 18	-	-
Human papillomavirus type 16	-	-
Chlamydophila pneumoniae	-	-
Neisseria meningitides	-	-
Enterococcus faecalis	-	-
Klebsiella pneumoniae carbapenemase	-	-
Enterobacter aerogenes	-	-
Enterobacter cloacae	-	-
Enterococcus avium	-	-
Staphylococcus aureus	-	-
sallmonela spp.	-	-
Klebsiella pneumoniae	-	-
Chlamydia trachomatis	-	-
Herpes simplex virus type 2	-	-
Ureaplasma parvum	-	-
Ureaplasma urealyticum	-	-
Clamydia psitachi	-	-
Mycoplasma genitalium	**+**	-
Mycoplasma pneumoniae	-	-
E.coli	-	-
Candida glabrata	-	-
Helicobacter pylori	-	-
Mycoplasma hominis	-	**+**

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Symbols for IVD Components and Reagents

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