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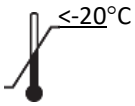
savyon DIAGNOSTICS

savvy·gen

H. pylori & Antibiotic Resistance

REF **621-01**

Test kit for 96 determinations



For Professional Use Only IVD

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Intended Use

The Savvygen™ H. pylori & Antibiotic Resistance is a qualitative test for the identification of *H. pylori* bacteria and a panel of 3 mutations related to its antibiotic resistance to Clarithromycin from stool samples of symptomatic patients.

For *in-vitro* professional diagnostic use.

Background

Helicobacter pylori (*H. pylori*) are common gastrointestinal bacteria closely associated with the incidence of chronic gastritis, peptic ulcers, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer. Despite the ongoing efforts to eradicate the disease etiology the prevalence of the *H. pylori* has remained high all over the world. The importance of *H. pylori* in the pathogenesis of gastric diseases and the need to eradicate it to prevent gastric cancer is well established. Numerous publications show that *H. pylori* eradication may rapidly decrease active inflammation in the gastric mucosa, prevent progression toward precancerous lesions and reverse gastric atrophy before the development of intestinal metaplasia. It has been clearly demonstrated that the earliest possible eradication of the bacterium is highly beneficial in many aspects. Evidently, during recent decades, the rate of antibiotic resistance (particularly to clarithromycin) has rapidly increased in most countries around the world. The most common resistance of *H. pylori* is to three antibiotics: clarithromycin, metronidazole or levofloxacin, while the prevalence of resistance to amoxicillin, tetracycline, rifabutin and furazolidone has remained low. Antibiotic resistance is the most important factor leading to the failure of eradication regimens, and therefore it is crucial to know in a timely manner the antibiotic resistance in individual patients, as well as the resistance pattern in the regional level of a population. The current product provides detection of the *H. pylori* and of the most abundant antibiotic resistance, i.e., to clarithromycin. prior to the first line of treatment. The diagnostic test is intended to enable the healthcare provider to offer the optimal and effective treatment in terms of using the right antibiotics in the desired combination in the earliest possible time, and by that facilitating bacterial eradication, preventing unnecessary use of antibiotics, shortening healing time, reducing patient suffering due to repeated empirical treatments, and reducing healthcare costs.

Molecular testing represents an attractive alternative to culture-based methods and has been recommended by the Maastricht Consensus guidelines for detection of *H. pylori* and clarithromycin resistance when standard culture and sensitivity testing are unavailable. Single point mutations (most common A2146C, A2146G and A2147G, formerly known as A2142C, A2142G and A2143G respectively[5]) within the *H. pylori* rrl gene that encodes the 23S ribosomal subunit are known to be associated with clarithromycin resistance[1-4]. Detection of these mutations is the ground of defining antibiotic resistance to Clarithromycin in the current test.

Principles of the Procedure

The Savvygen™ H. pylori & Antibiotic Resistance is designed for the identification of H. pylori and its antibiotic resistance to Clarithromycin in human stool specimens or culture to aid in the assessment of infections caused by this bacterium and the way to treat it.

The Savvygen™ H. pylori & Antibiotic Resistance is based on amplification of highly specific conserved fragments in the 23s gene encoded by the *H. pylori* genome. The assay is based on endpoint genotyping analysis that uses hydrolysis probes for single-nucleotide polymorphism (SNP) genotyping. A

fluorophore/quencher dual-labeled probe is annealed to an internal specific sequence recognizing the point mutations associated with the antibiotic resistance (Table 1). Upon primer elongation, Taq DNA Polymerase displaces and hydrolyzes the probe, thus releasing and activating the fluorophore.
Fluorescence data are collected using PCR. To identify genotypes, only endpoint fluorescence intensities of the two reporter dyes are used. Wild-type sequences are identified by the CAL Fluor Orange 560 probe while mutant sequences by the FAM probe. Whether the detected *H. pylori* bears the resistance to Clarithromycin is dependent upon the ratio between the intensities of the fluorescent signals of the two probes. Thus, if the CAL Fluor Orange 560 /FAM is higher than the cutoff, the infection consists primarily of wild-type bacteria, while in case the ratio is lower than the designated cutoff, then the resistant to Clarithromycin is prominent.

Table 1. *H. pylori* associated mutations detected in Savvygen™ H. pylori & Antibiotic Resistance test

Antibiotic resistance	Point mutation related
Clarithromycin	A2143G A2142C A2142G

Materials/ Reagents Provided

Product Description	Contents
Savvygen™ H. pylori & Antibiotic Resistance ***96 reactions*. REF# 621-01**	3 x HP Mix (550µl) 1x Positive Control mix (100µl) 1x Negative Control (500µl)

Additional Equipment and Material Required

- Centrifuge for 1.5 mL tube.
- Micropipettes (0.5-20 µL, 20-200 µL).
- Powder-free disposal gloves
- Vortex
- Real Time PCR instrument

***Note*: Savvygen™ H. pylori & Antibiotic Resistance test has been designed and validated for use on Bio-Rad CFX96 Touch™ Real-Time PCR Detection System (Biorad) only.**

Transport and Kit Storage

The Savvygen™ kits are shipped and stored at -20ºC until expiration date as stated in the label.

Precautions

Amplification technologies can amplify target nucleic acid sequences over a billion-fold and provide a means of detecting very low concentrations of target. Care must be taken to avoid contamination of samples with target molecules from other samples, or amplicons from previous amplifications. Follow these recommendations to help control contamination.

1. 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.
2. 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.
3. Use disposable containers, disposable barrier pipette tips, disposable bench pads, and disposable 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 amplicon. Use the bleach solution to wipe down equipment and bench areas, as well as to treat drains used to dispose of liquid waste.
5. Use negative controls to monitor for possible contamination during reaction setup. If reagent contamination is detected, dispose of the suspect reagents.
6. 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.
7. Do not use after the expiration date stated on the box.
8. Dispose of all waste materials in accordance with local rules.

Test Procedure

Specimen Collection, Processing and DNA Extraction

Specimens received in the laboratory should be processed upon arrival. In case of delay, store specimens refrigerated (2-8°C) for up to 48 hours or at -20°C for a longer period. Store purified nucleic acids at ≤ – 20°C.

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:

- Savvygen Stool NA Extraction kit (REF; 690-01)
- DNeasy PowerSoil Pro kit (Qiagen)

Note: Take ~250mg of stool sample and procced according to the manufacture instructions.

Preparing reaction wells

The following will be performed in an amplicon-free area.

1. Thaw the HP Mix, Positive Control and Negative Control tubes.
2. Before use, mix the HP Mix by inverting the tube 3-5 times and then do a short spin down. **Do not vortex!**

Note: *Each HP Mix vial is good for up to 2 freeze/thaw cycles. After 3 uses the vial should be disposed according to good laboratory practice.*

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

3. Pipette 15µl from the HP Mix into separate wells
4. Pipette 10µl of DNA sample into each sample well containing the HP Mix
5. Pipette 10µl of Positive Control into each positive control well
6. Pipette 10µl of Negative Control into each negative control well
7. Cover the wells with the suitable caps/seal, spin down briefly.

- ***In case of using automation,***
  - ***place the HP Mix vial m Positive and Negative control on board according to the robot instruction.***
  - ***Seal the plate and place on CFX-96 for analysis***

PCR Protocol Program setting

Set your CFX96 thermocycler according to **Table 2**.

The Savvygen™ H. pylori & Antibiotic Resistance test is designed for detecting FAM and Cal Fluor Orange 560 (VIC/HEX/JOE channel). The mutation-specific probes are labelled with FAM, while the wild type-specific probes are labelled with Cal Fluor Orange 560. The probe for the Extraction Control is labeled with the Cal Red 610 fluorophore.

Target	Dye	Channel
Wild type	CAL Fluor Orange 560	HEX
Mutation	FAM	FAM
Extraction Control	Cal Red 610	Cal Red 610

Table 2. Real time-PCR profile

Step	Temperature (ºC)	Time	Cycles
Polymerase	95	1 min	1
Denaturation Annealing/Extension	95 58 *	5 sec 30 sec	50

Note: *Set the fluorescence data collection during the extension step (*).*

Performing qPCR.

1. Place the plate/strips in the Real-Time PCR instrument.
2. Start the run.

Analysis of Results

Use the Savvycheck Software analysis for results interpretation

Limitations of the test

- The results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms and other diagnostic tests.
- Savvygen™ H. pylori & Antibiotic Resistance assay was validated only with stool and culture samples. The use of other types of samples has not been established.
- Savvygen™ H. pylori & Antibiotic Resistance assay was validated only on Bio-Rad CFX96 Touch™ Real-Time PCR Detection System (Biorad). The use of other Real-Time PCR instruments has not been established.
- Savvygen™ H. pylori & Antibiotic Resistance assay was validated only with samples extracted using DNeasy PowerSoil Pro kit (Qiagen) and Savvygen Stool NA Extraction kit (REF; 690-01)
- system. The use of other extraction methods has not been established.
- Error results may occur from improper sample collection, handling, storage, technical error, sample mix-up or because the number of organisms in the sample is below the analytical sensitivity of the test. Other factors are the presence of Real Time amplification inhibitors or other types of interference and failure to follow the manufacturer´s instructions and procedures.
- Negative results do not preclude infection with the *H. pylori* and should not be the sole basis of a patient treatment/management decision. Consider the collection of multiple specimens from the same patient at different time points, which may increase the probability of detecting the bacteria.
- The presence of PCR inhibitors may cause invalid results.
- A false positive result is possible due to contamination with 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 samples result is "Suspicious in Clarithromycin resistance H. pylori", then the sample should be repeated from NA extraction.
- If a certain sample result is "Invalid", then the sample should be repeated from NA extraction.

Quality Control

In order to confirm the appropriate performance of the molecular diagnostic technique, a Positive Control and a Negative Control (RNase/DNase Free Water) must be included in each assay to interpret the results correctly. An Extraction Control (16s bacterial gene) validates the whole process of the extraction and amplification process.

Performance Characteristics

Clinical sensitivity and specificity

Clinical performance characteristics of the Savvygen™ H. pylori & Antibiotic Resistance test were assessed by evaluation of retrospective (frozen) stool specimens. These specimens are consisted of residual anonymized stools collected and processed in clinical laboratories, which was verified by CE approved methods. The performance of the Savvygen™ H. pylori & Antibiotic Resistance test is presented in table 3(a,b):

Table 3a. *H. Pylori* identification

		Reference Method		
		Positive	Negative	Total
Savvygen™ H. pylori & Antibiotic Resistance	Positive	199	0	199
	Negative	5	45	50
	Total	204	45	249

Sensitivity: 199/204=97.5% , (P=0.99, Lowe 95%Confidence Interval (CI)= [0.954 – 0.997])
Specificity: 45/45=100% , (P=0.99, Lowe 95%Confidence Interval (CI)= [0.979 – 1])

Table 3b. *H. Pylori* Clarithromycin Resistance identification

		Reference Method		
		Positive	Negative	Total
Savvygen™ H. pylori & Antibiotic Resistance	Positive	38	0	38
	Negative	2*	214	216
	Total	40	214	254

***The sample was verified by sequencing as containing both H. Pylori and H. Pylori Clarithromycin Resistance*
Sensitivity: 38/40=95%
Specificity: 214/214=100%

Analytical sensitivity and specificity

Analytical sensitivity

Serial dilutions of H. pylori DNA sample with known copy number were tested in three different batches in consecutive experiments. Six replicates of each dilution were tested per batch and the limit of detection (LOD) was set accordingly to the last dilution in which all replicates were identified (1.5 cp/µl).

Table 4: Analytical sensitivity experiment

Copies/µl	Copies/reaction	Replicates tested	Replicates detected	Detection success rates
2.17	21.7	18	18/18	100%
1.5	15	18	18/18	100%
1.2	12	18	13/18	83.3%
1	10	18	11/18	61%

Analytical specificity

In order to detect possible cross-reactions of the Savvygen™ H. pylori & Antibiotic Resistance, samples positive for different enteric or flora pathogens present in the intestine were tested. None of the tested pathogens revealed a positive result.

Table 5: Cross-Reactivity experiment

Pathogen	HP Mix
Helicobacter pylori	+
Salmonella Enteritidis	-
Shigella Spp.	-
Cryptosporidium parvum	-
Enterococcus faecalis	-
Enterococcus faecium	-
Klebsiella pneumoniae	-
Candida glabrata	-
Candida tropicalis	-
Candida krusei	-
Candida albicans	-
Giardia lamblia	-
Dientamoeba fragilis	-
Blastocystis hominis	-
Methicillin-resistant Staphylococcus aureus	-
Staphylococcus epidermidis	-
Staphylococcus capitis	-
Staphylococcus warneri	-
Staphylococcus cohnii	-
Campylobacter jejuni	-
Clostridioides difficile	-
Entamoeba histolytica	-

Technical Support:

Please refer to the phone number of your local distributor as listed on the test's box or contact Savyon Diagnostics at info@savyondx.com

Bibliography

1. Denise E Brennan et al. **Molecular detection of Helicobacter pylori antibiotic resistance in stool vs biopsy samples.** World journal of Gastroenterology. November 7, 2016,Volume 22,Issue 41.

2. Malfertheiner P et al. **Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report**. Gut 2012, 61: 646-664**.**

3. Smith SM et al. **Antimicrobial susceptibility testing for Helicobacter pylori in times of increasing antibiotic resistance**. World J Gastroenterol. 2014, 20, 9912-9921.

4. Mégraud F et al. **Molecular Approaches to Identify Helicobacter pylori Antimicrobial Resistance.** Gastroenterol Clin North Am. 2015, 44, 577-596.

5. González-Hormazábal P et al. **Prevalence of Helicobacter pylori antimicrobial resistance among patients recruited in endoscopy units in Santiago, Chile.** Arch Med Res. 2021 July ; 52(5): 529–534.

Symbols for IVD Components and Reagents

	Manufacturer		Use by		Keep Dry
LOT	Lot number		Temperature limitation		Consult instructions for use
REF	Catalogue number		Contains sufficient for <n> test	DIL	Buffer (sample diluent)