IVD For *in Vitro* Diagnostic Use

CE

Neisseria gonorrhoeae Real-TM

Handbook

Real Time PCR kit for qualitative detection of Neisseria gonorrhoeae

REF B5-100FRT

Σ **100**

NAME

Neisseria gonorrhoeae Real-TM

INTRODUCTION

STDs (sexually transmitted diseases) refer to a variety of bacterial, viral and parasitic infections that are acquired through sexual activity. Some STDs, such as syphilis and gonorrhea, have been known for centuries — while others, such as HIV, have been identified only in the past few decades. STDs are caused by more than 25 infectious organisms. As more organisms are identified, the number of STDs continues to expand. Common STDs include: chlamydia, gonorrhea, herpes, HIV, HPV, syphilis, ureaplasma, mycoplasma, gardnerella and trichomoniasis.

The development of tests based on nucleic acid amplification technology has been the most important advance in the field of STD diagnosis. Because nucleic acid amplification is exquisitely sensitive and highly specific, it offers the opportunity to use noninvasive sampling techniques to screen for infections in asymptomatic individuals who would not ordinarily seek clinical care.

INTENDED USE

Kit **Neisseria gonorrhoeae Real-TM** is a test for the qualitative detection of *Neisseria gonorrhoeae* in the urogenital swabs, urine, prostatic liquid and other biological materials.

PRINCIPLE OF ASSAY

Kit **Neisseria gonorrhoeae Real-TM** is based on two major processes: isolation of DNA from specimens and Real Time amplification. *Neisseria gonorrhoeae* DNA is extracted from the specimens, amplified using Real-Time amplification and detected fluorescent reporter dye probes specific for *Neisseria gonorrhoeae* DNA and Internal Control. Internal Control (IC) serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition. IC is detected in a channel other than the *Neisseria gonorrhoeae*.

MATERIALS PROVIDED

Real Time PCR kit (B5-100FRT)

"**Neisseria gonorrhoeae Real-TM**"

- **PCR-mix-1-FL**, 1,2 ml;
- **PCR-mix-2**, 2 x 0,3 ml;
- **TaqF Polymerase**, 2 x 0,03 ml;
- **Pos C+**, 0,2 ml;
- **Negative Control C-***, 1,2 ml;
- **Internal Control IC****, 1,0 ml;
- **DNA-buffer**, 0,5 ml;

Contains reagents for 110 tests.

* *must be used in the isolation procedure as Negative Control of Extraction.*
** *add 10 µl of Internal Control during the DNA isolation directly to the sample/lysis mixture.*

MATERIALS REQUIRED BUT NOT PROVIDED

- Transport medium.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers up to 200 µl.
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Real Time PCR instrument.
- Disposable polypropylene microtubes for PCR or PCR-plate.
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

QUALITY CONTROL

In accordance with Sacace's ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

PRODUCT USE LIMITATIONS

All reagents may exclusively be used in in vitro diagnostics. Use of this product should be limited to personnel trained in the techniques of DNA amplification (UNI EN ISO 18113-2:2012). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

WARNINGS AND PRECAUTIONS

IVD

***In Vitro* Diagnostic Medical Device**

For *In Vitro* Diagnostic Use Only

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local authorities' regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- 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.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

STORAGE INSTRUCTIONS

The components of **Neisseria gonorrhoeae Real-TM** PCR kit are to be stored at the temperature 2–8 ºC, except for **TaqF Polymerase** and **PCR-mix-2** that need to be stored at -16°C or below when not in use. The kit can be shipped at 2-8°C and should be stored at 2-8°C and -16°C or below immediately on receipt.

STABILITY

Neisseria gonorrhoeae Real-TM is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Neisseria gonorrhoeae Real-TM can analyze DNA extracted from:

- *cervical, urethral swabs:* insert the swab into the nuclease-free 1,5 ml tube and add 0,2 ml of Transport medium. Vigorously agitate swabs in medium for 15-20 sec.
- *urine sediment* (use the first part of the stream);
- *prostatic liquid* stored in “Eppendorf” tube;
- *seminal liquid: t*ransfer about 30 µl of seminal liquid to a polypropylene tube (1,5 ml) and add 70 µl of sterile saline solution;

It is recommended to process samples immediately after collection. Store samples at 2–8 °C for no longer than 24 hours, or freeze at –20/80°C. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

DNA ISOLATION

Any commercial RNA/DNA isolation kit, if IVD-CE validated for the specimen types indicated herein at the "SAMPLE COLLECTION, STORAGE AND TRANSPORT" paragraph, could be used.

Sacace Biotechnologies recommends to use the following kits:

- ⇨ **DNA-Sorb-A** (Sacace, REF K-1-1/A);
- ⇨ **SaMag STD DNA Extraction kit** (Sacace, REF SM007).

Please carry out DNA extraction according to the manufacture's instruction. Add 10 µl of Internal Control during DNA isolation procedure directly to the sample/lysis mixture.

(Note: Sacace Internal Control is the same for all urogenital infection Real Time kits)

PROTOCOL:

1. Prepare required quantity of reaction tubes for samples (N) and controls (N+2).
2. Prepare in the new sterile tube for each sample **10*(N+1) µl** of **PCR-mix-1-FL**, **5,0*(N+1)** of **PCR-mix-2** and **0,5*(N+1)** of **TaqF Polymerase.** Vortex and centrifuge for 2-3 sec.
3. Add to each tube **15 µl** of **Reaction Mix** and **10 µl** of **extracted DNA** sample to appropriate tube. Mix by pipetting.
4. Prepare for each panel 2 controls:
   - add **10 µl** of **DNA-buffer** to the tube labeled Amplification Negative Control;
   - add **10 µl** of **Positive Control C+** to the tube labeled Amplification Positive Control;
5. Insert the tubes in the thermalcycler.

Amplification

1. Create a temperature profile on your instrument as follows:

	Rotor-type Instruments[1]			Plate- or modular type Instruments[2]		
Step	***Temperature, °C***	***Time***	**Repeats**	***Temperature, °C***	***Time***	**Repeats**
1	**95**	15 min	1	**95**	15 min	1
2	**95**	5 s	5	**95**	5 s	5
	60	20 s		**60**	20 s	
	72	15 s		**72**	15 s	
3	**95**	5 s	40	**95**	5 s	40
	60	20 s *fluorescent signal detection*		**60**	30 s *fluorescent signal detection*	
	72	15 s		**72**	15 s	

[1] For example Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen)
[2] For example, SaCycler-96™ (Sacace), CFX96/iQ5™ (BioRad); Mx3005P™ (Agilent), ABI® 7300/7500/StepOne Real Time PCR (Applied Biosystems), SmartCycler® (Cepheid), LineGeneK® (Bioer)

Fluorescence is detected at the 2nd step of Cycling 2 stage (60 °C) in FAM/Green and JOE/Yellow/Hex/Cy3 fluorescence channels.

Neisseria gonorrhoeae is detected on the FAM (Green) channel, *IC DNA* on the JOE(Yellow)/HEX/Cy3 channel.

INSTRUMENT SETTINGS

Rotor-type instruments

Channel	Calibrate/Gain Optimisation…	Threshold	More Settings/ Outlier Removal	Slope Correct
FAM/Green	*from 5 Fl to 10 Fl*	*0.1*	*0 %*	*Off*
JOE/Yellow	*from 4 Fl to 8 Fl*	*0.1*	*5 %*	*Off*

Plate-type instruments

The threshold line should cross only sigmoid curves of signal accumulation of positive samples and should not cross the baseline; otherwise, the threshold level should be raised. Set the threshold at a level where fluorescence curves are linear and do not cross curves of the negative samples.

Boundary value of the cycle threshold, Ct

Sample	Channel for fluorophore	Ct boundary value	
		Rotor-type instruments	Plate-type instruments
C+	FAM/Green	33	36
	JOE/Yellow/Hex/Cy3	30	33
Clinical samples, C-	JOE/Yellow/Hex/Cy3	30	33

DATA ANALYSIS

The fluorescent signal intensity is detected in two channels:

– The signal from the *Neisseria gonorrhoeae* DNA amplification product is detected in the FAM/Green channel;

– The signal from the Internal Control amplification product is detected in the JOE/Yellow/HEX/Cy3 channel.

Interpretation of results

The results are interpreted by the software of the instrument by the crossing (or not crossing) of the fluorescence curve with the threshold line.

Principle of interpretation:

— *Neisseria gonorrhoeae* DNA is **detected** in a sample if its Ct value is present in the FAM channel. The fluorescence curve should cross the threshold line in the area of exponential fluorescence growth.

— *Neisseria gonorrhoeae* DNA is **not detected** in a sample if its Ct value is absent in the FAM channel (fluorescence curve does not cross the threshold line) while the Ct value in the JOE channel is less than 33.

— The result is **invalid** if the Ct value of a sample in the FAM channel is absent while the Ct value in the JOE channel is either absent or greater than the specified boundary value (Ct > 33). It is necessary to repeat the PCR analysis of such samples.

The result of analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (Table 1).

Table 1. Results for controls

Control	Stage for control	Ct channel Fam	Ct channel Joe	Interpretation
NCE	DNA isolation	NEG	POS	Valid result
NCA	Amplification	NEG	NEG	Valid result
C+	Amplification	POS	POS	Valid result

QUALITY CONTROL PROCEDURE

A defined quantity of Internal Control (IC) is introduced into each sample and control at the beginning of sample preparation procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

A negative control of extraction (NCE), negative amplification control (NCA), positive amplification control (C+) are required for every run to verify that the specimen preparation, the amplification and the detection steps are performed correctly.

If the controls are out of their expected range (see table Results for Controls), all of the specimens and controls from that run must be processed beginning from the sample preparation step.

SPECIFICATIONS

Sensitivity

The analytical sensitivity of **Neisseria gonorrhoeae Real-TM** PCR kit is specified in the table below.

Clinical material	DNA extraction kit	Analytical sensitivity, GE/ml*
Urogenital swabs	DNA-sorb-A	5×10^2
Urine	DNA-sorb-A	1×10^3

** Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.*

Specificity

The analytical specificity of **Neisseria gonorrhoeae Real-TM** PCR kit is ensured by selection of specific primers and probes as well as by selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. There were no nonspecific responses during examination of human DNA as well as DNA panel of the following microorganisms: *Gardnerella vaginalis, Lactobacillus spp., Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae, Candida albicans, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma genitalium, Neisseria flava, Neisseria subflava, Neisseria sicca, Neisseria mucosa, Chlamydia trachomatis, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV* type 1 and 2, *CMV,* and *HPV.*

Target region

Channel for fluorophore	FAM	JOE
DNA-target	***Neisseria gonorrhoeae DNA***	**Internal Control IC DNA**
Target gene	*gene 16S rRNA*	Artificially synthesized sequence

TROUBLESHOOTING

1. Weak or no signal of the IC (Joe/Hex/Cy3 channel) for the Negative Control of extraction.
    - The PCR was inhibited.
        - ⇒ Make sure that you use a recommended DNA extraction method and follow to the manufacturer's instructions.
        - ⇒ Re-centrifuge all the tubes before pipetting of the extracted DNA for 2 min at maximum speed (12000-16000 g) and take carefully supernatant. Don't disturb the pellet, sorbent inhibit reaction.
    - The reagents storage conditions didn't comply with the instructions.
        - ⇒ Check the storage conditions
    - Improper DNA extraction.
        - ⇒ Repeat analysis starting from the DNA extraction stage
    - The PCR conditions didn't comply with the instructions.
        - ⇒ Check the PCR conditions and select for the IC detection the fluorescence channel reported in the protocol.
    - The IC was not added to the sample during the pipetting of reagents.
        - ⇒ Make attention during the DNA extraction procedure.
2. Weak or no signal of the Positive Control.
    - The PCR conditions didn't comply with the instructions.
        - ⇒ Check the amplification protocol and select the fluorescence channel reported in the manual.
3. Fam (Green) signal with Negative Control of extraction.
    - Contamination during DNA extraction procedure. All samples results are invalid.
        - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol.
        - ⇒ Use only filter tips during the extraction procedure. Change tips between tubes.
        - ⇒ Repeat the DNA extraction with the new set of reagents.
4. Any signal with Negative Control of PCR (DNA-buffer).
    - Contamination during PCR preparation procedure. All samples results are invalid.
        - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents.
        - ⇒ Pipette the Positive control at last.
        - ⇒ Repeat the PCR preparation with the new set of reagents.

KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
IVD	For *in Vitro* Diagnostic Use	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**C–**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control

* SaCycler™ is a registered trademark of Sacace Biotechnologies
* CFX™ and iQ5™ are registered trademarks of Bio-Rad Laboratories
* Rotor-Gene™ is a registered trademark of Qiagen
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