Sacace BIOTECHNOLOGIES

IVD For *in Vitro* Diagnostic Use

CE

Neisseria gonorrhoeae Real-TM Quant

Handbook

Real Time PCR Kit for quantitative detection of *Neisseria gonorrhoeae*

REF B204-100FRT

Σ 100

NAME

Neisseria gonorrhoeae Real-TM Quant

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, mycoplasma, herpes, HIV, HPV, syphilis, 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 Quant** is a test for the quantitative detection of *Neisseria gonorrhoeae* in the whole blood, tissue, urogenital swabs, urine, prostatic liquid and other biological materials.

PRINCIPLE OF ASSAY

Kit **Neisseria gonorrhoeae Real-TM Quant** is based on two major processes: isolation of DNA from specimens and Real Time amplification. In real-time PCR, the fluorescent signal is generated from the presence of an olygonucleotide probe specific for target DNA sequence. The probe contains a fluorescent dye molecule on its 5' end and a quencher molecule on its 3' end. The probe hybridizes with one of the chains of the amplified fragment. During synthesis of a complementary chain, Taq DNA polymerase cleaves the probe due to its 5'-3' nuclease activity. As a result, the fluorescent dye molecule becomes separated from the quencher, and the total fluorescence of reaction volume increases in direct proportion to the number of amplicon copies synthesized during PCR. The fluorescent signal is measured in each cycle of reaction, and the threshold cycle value is determined from the obtained curve. The threshold cycle is proportional to the initial number of DNA copies in a sample, and its value allows quantitative comparisons of analyzed and control samples.

In **Neisseria gonorrhoeae Real-TM Quant** kit there are 2 independent reactions running in parallel in each tube: the first reaction allows to detect and to quantify the specific fragment of *Neisseria gonorrhoeae* (Joe/Yellow/Cy3/HEX channel) and the second reaction detects β-actin gene (Fam/Green channel) present in all samples obtained from cells and allows not only to control all analysis steps, but also to estimate sample handling and storage.

MATERIALS PROVIDED

Module No.1: Real Time PCR kit (B204-100FRT)

- **PCR-mix-1 FRT**, 4 x 0,55 ml
- **Taq Polymerase**, 2 x 0,03 ml
- **Pos C+** (N. gonorrhoeae + β-actin)*, 0,2 ml
- **Standards:**
  - **QS1** (10^7 copies/sample N. gonorrhoeae + β-actin), 0,2ml
  - **QS2** (10^5 copies/sample N. gonorrhoeae + β-actin), 0,2ml
  - **QS3** (10^3 copies/sample N. gonorrhoeae + β-actin), 0,2ml
- **Negative Control C-****, 0,2 ml

** must be used in the amplification procedure as Positive Control of Amplification.*
*** must be used in the amplification procedure as Negative Control of Amplification.*

MATERIALS REQUIRED BUT NOT PROVIDED

- DNA isolation kit
- Desktop microcentrifuge for “eppendorf” type tubes
- Vortex mixer
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator, Freezer
- Real Time Thermal cycler
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Tube racks

STORAGE INSTRUCTIONS

Neisseria gonorrhoeae Real-TM Quant must be stored at -20°C. The **Neisseria gonorrhoeae Real-TM Quant** kit can be shipped at 2-8°C but should be stored at -20°C immediately on receipt.

STABILITY

Neisseria gonorrhoeae Real-TM Quant 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.

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.

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.

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.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Neisseria gonorrhoeae Real-TM Quant can analyze DNA extracted from:

- *cervical, urethral, conjunctival 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* : collect 10-20 ml of first-catch urine in a sterile container. Centrifuge for 30 min at 3000 x g, carefully discard the supernatant and leave about 200 µl of solution. Resuspend the sediment. Use the suspension for the DNA extraction.
- *prostatic liquid* stored in “Eppendorf” tube;
- *seminal liquid:* maintain semen for 40 min in darkness until liquefaction. Use 100 µl for the DNA extraction.

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 the DNA extraction according to the manufacturer’s instructions.

PROTOCOL:

1. Prepare required quantity of reaction tubes (or PCR plate) for samples and controls.
2. Prepare in the new sterile tube for each sample **15*N µl** of **PCR-mix-1-FRT** and **0,5*N µl** of **Taq DNA Polymerase.** Vortex and centrifuge briefly.
3. Add to each tube **15 µl** of **Reaction Mix**.
4. Add **10,0 µl** of **extracted DNA** sample to appropriate tube with Reaction Mix.
5. Prepare for each run 3 standards, 1 Pos Control and 1 Neg Control:
   - add **10,0 µl** of **Quantitation Standards** (QS1, QS2, QS3) into 3 labeled tubes;
   - add **10,0 µl** of **Negative Control** to the tube labeled PCR Negative Control;
   - add **10,0 µl** of **Pos C+** to the tube labeled PCR Pos Control.
6. Insert the tubes in the thermalcycler.

The results are interpreted through the presence of crossing of fluorescence curve with the threshold line.

N. gonorrhoeae is detected on Joe/Yellow/Cy3/HEX and β-actin gene on Fam/Green channel.

Create a temperature profile on your Real-time instrument as follows:

	Rotor type instruments[1]				Plate type or modular instruments[2]			
Stage	**Temp, °C**	**Time**	**Fluorescence detection**	**Cycle repeats**	**Temp,°C**	**Time**	**Fluorescence detection**	**Cycle repeats**
Hold	94	300s	–	1	95	5 min	–	1
	61	50 s	FAM(Green), JOE(Yellow)	50	62	60 s	FAM, JOE/HEX/Cy3,	50
	94	20 s	–		95	20 s	–	

[1] For example Rotor-Gene™ 3000/6000 (Corbett Research, Australia)
[2] For example, *SaCycler-96™ (Sacace), CFX96™*/ iQ5™/iQ iCycler™ (BioRad, USA); Mx3000P/Mx3005P™ (Stratagene, USA), Applied Biosystems® 7300/7500 Real Time PCR (Applera), SmartCycler® (Cepheid)

RESULTS INTERPRETATION

The results are interpreted through the presence of crossing of fluorescence curve with the threshold line. To set threshold put the line at such level where curves of fluorescence are linear.

- *Neisseria gonorrhoeae* DNA amplification is detected on JOE(Yellow)/HEX/Cy3 channel;
- β-actin gene DNA amplification is detected on FAM (Green) channel.

Qualitative analysis

Results are accepted as relevant if positive and negative controls of amplification and extraction are passed.

Results for controls

Control	Stage for control	Ct FAM (Green)	Ct JOE(Yellow)/HEX/Cy3	Interpretation
NCE	DNA isolation, PCR	–	–	OK
Pos C+	PCR	Pos	Pos	OK
NCA	PCR	–	–	OK

- The sample is considered to be **positive** for *Neisseria gonorrhoeae* if in the channel JOE (Yellow)/HEX/ Cy3 the value of **Ct** is different from zero (Ct<40);
- The sample is considered to be **uncertain** for *Neisseria gonorrhoeae* if its Ct value is more than 40 on FAM (Green) and/or JOE(Yellow)/HEX/Cy3 channels. Additional double study of this sample should be conducted;
- Specimens with Ct < 33 in the channel FAM (Green) and absent fluorescence signal in the JOE(Yellow)/HEX/Cy3 channel are interpreted as **negative**.
- Specimens with absent signal in the FAM (Green) and JOE(Yellow)/HEX/Cy3 channels are interpreted as **invalid**.

Quantitative analysis

For each control and patient specimen, calculate the concentration of *Neisseria gonorrhoeae* DNA in 10^5 cells using the following formula:

$$\frac{\textbf{\textit{N. gonorrhoeae} DNA copies/reaction (JOE(Yellow)/HEX/Cy3 channel)}}{\textbf{β-actin DNA copies/reaction (FAM (Green) channel)}} \textbf{ x } 2*10^6 \textbf{ = copies } \textit{N. gonorrhoeae} \textbf{ /}10^5 \textbf{ cells}$$

For each control and patient specimen, calculate the concentration in logarithms of *N. gonorrhoeae* DNA in 10^5 cells using the following formula:

lg [*N. gonorrhoeae* DNA copies/ β-actin DNA copies x $2*10^6$] = lg (copies *N. gonorrhoeae* DNA/10^5 cells)

The results can be calculated manually or using Excel tables. To do this copy the names of the samples and insert them in the first column (Column A). Copy the concentrations of ***N. gonorrhoeae*** DNA from the channel JOE(Yellow)/HEX/Cy3 and paste in the second column of Excel table (Column B). Copy the concentrations of **β-actin** from the channel FAM(Green) and paste in the third column of Excel table (Column C). Insert in the column D the formula D=LOG (B/C*2000000): lg values will appear. In E column put the formula E=B/C*2000000: the values in copies/10^5 cells will appear.

Name	Calc Conc (copies/reaction) Joe(Yellow)/HEX/ Cy3	Calc Conc (copies/reaction) Fam(Green)	lg *N. gonorrhoeae* /10^5	copies *N. gonorrhoeae* /10^5
A	B	C	D	E
1	46980	24040	6,59	3908486
2	5029	9150	6,04	1099235
3		4136	n/a	0
4		14450	n/a	0
5	93905	48107	6,59	3904006
QS1	9950000	11100000	6,25	1792793
QS2	119000	105000	6,36	2266667
QS3	1110	1210	6,26	1834711
Neg PCR				

PERFORMANCE CHARACTERISTICS

Analytical specificity

The analytical specificity of the primers and probes was validated with negative samples. They did not generate any signal with the specific *N. gonorrhoeae* primers and probes. The specificity of the kit **Neisseria gonorrhoeae Real-TM** was 100%. The potential cross-reactivity of the kit **Neisseria gonorrhoeae Real-TM** was tested against the group control. It was not observed any cross-reactivity with other pathogens.

Analytical sensitivity

The kit **Neisseria gonorrhoeae Real-TM** allows to detect *N. gonorrhoeae* DNA in 100% of the tests with a sensitivity of not less than 500 copies/ml. The detection was carried out on the control standard and its dilutions by negative sample.

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	**NCE**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control

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