Sacace BIOTECHNOLOGIES

CE

IVD For *in Vitro* Diagnostic Use

For Professional Use Only

NHS Meningitidis Real-TM Handbook

Real time PCR kit for detection of *Neisseria meningitidis, Haemophilus influenzae* and *Streptococcus pneumoniae*

REF B25-50FRT

Σ 50

NAME

NHS Meningitidis Real-TM

INTENDED USE

Kit **NHS Meningitidis Real-TM** is a Real-Time test for the detection and differentiation of *Neisseria meningitidis, Haemophilus influenzae* and *Streptococcus pneumoniae* in the biological materials. DNA is extracted from specimens, amplified using RT-amplification and detected using fluorescent reporter dye probes specific for *N.meningitidis, H.influenzae, S.pneumoniae* DNA and IC (Internal Control).

PRINCIPLE OF ASSAY

NHS Meningitidis Real-TM Test is based on three major processes: isolation of DNA from specimens, Real Time amplification of the DNA and *NHS Meningitidis* detection by the polymerase chain reaction (PCR) based on the amplification of pathogen genome specific region using specific primers and detection via fluorescent dyes. These dyes are linked with probes of oligonucleotides which bind specifically to the amplified product. The real-time PCR monitoring of fluorescence intensities allows the accumulating product detection without reopening of reaction tubes after the PCR run. **NHS Meningitidis Real-TM** PCR kit is a qualitative test which contain the Internal Control (IC). It must be used in the isolation procedure in order to control the process of each individual sample extraction and serves also to identify possible reaction inhibition.

MATERIALS PROVIDED

"Controls"

- **Negative Control C- ***, 1,2 ml;
- **Internal Control (IC) ****, 1,0 ml;
- **Pos DNA *N. meningitidis* C+,** 0,1 ml;
- **Pos DNA *H.influenzae* C+,** 0,1 ml
- **Pos DNA *S.pneumoniae* C+,** 0,1 ml;
- **Pos IC C+,** 0,1 ml;
- **DNA-buffer**, 0,5 ml;

"NHS Meningitidis Real-TM": Real Time amplification

- **PCR-mix-1 *N. meningitidis*/IC**, 0,6 ml;
- **PCR-mix-1 *S. pneumoniae/ H. influenzae*** 0,6 ml;
- **PCR-mix-2**, 2 x 0,3 ml;
- **TaqF Polymerase**, 2 x 0,03 ml;

Contains reagents for 55 reactions

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

MATERIALS REQUIRED BUT NOT PROVIDED

Zone 1: sample preparation:

- DNA extraction kit.
- Transport medium.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers.
- Disposable polypropylene 1,5/2,0 ml tubes.
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 1,5/2,0 ml tubes.
- PCR Workstation.
- Real Time Thermal cycler.
- Disposable polypropylene microtubes for PCR.
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

STORAGE INSTRUCTIONS

"Controls" must be stored at 2-8°C.

"NHS Meningitidis Real-TM" must be stored at -20°C.

The kit can be shipped at 2-8°C but should be stored at 2-8°C and -20°C immediately on receipt.

STABILITY

NHS Meningitidis Real-TM test 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

1. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
4. Do not use a kit after its expiration date.
5. Dispose of all specimens and unused reagents in accordance with local regulations.
6. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.
7. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
8. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
9. Material Safety Data Sheets (MSDS) are available on request.
10. Use of this product should be limited to personnel trained in the techniques of DNA amplification.
11. PCR reactions are sensitive to contamination. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practice.
12. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the PCR and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

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

Sampling of biological materials for PCR-analysis, transportation, and storage are described in details in the handbook of the manufacturer. It is recommended that this handbook is read before beginning of the work.

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

NHS Meningitidis Real-TM can analyze DNA extracted from:

- *liquor* (ready for extraction)- 0,1 ml;

Specimens can be stored at +2-8°C for no longer than 12 hours, or frozen at -20°C to -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-B** (Sacace, REF K-1-1/B/100);
- ⇨ **DNA/RNA Prep** (Sacace, REF K-2-9);
- ⇨ **SaMag Bacterial DNA Extraction kit** (Sacace, REF SM006);

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

REAGENTS PREPARATION (REACTION VOLUME 25 µL):

Total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

1 Prepare required quantity of reaction tubes (2 tubes for each sample + Controls)

2 Prepare in a new sterile tube two **Reaction Mixes.**

- The Reaction Mix ***S. pneumoniae/ H. influenzae*:**

  10*(N+1) µl of **PCR-mix-1** ***S. pneumoniae/ H. influenzae***

  5.0*(N+1) µl of **PCR-mix-2**

  0.5*(N+1) µl of **TaqF Polymerase**

- The Reaction Mix ***N. meningitidis*/IC:**

  10*(N+1) µl of **PCR-mix-1** ***N. meningitidis*/IC**

  5.0*(N+1) µl of **PCR-mix-2**

  0.5*(N+1) µl of **TaqF Polymerase**

3 Vortex the tube, then centrifuge shortly. Add **15 µl** of prepared reaction mix into each appropriate tube.

4 Using tips with aerosol filter add **10 µl** of DNA samples obtained at the stage of DNA isolation and mix carefully by pipetting**.**

5 Prepare for each panel 3 controls:

- add **10 µl** of **DNA-buffer** to the tube labeled Amplification Negative Control;
- add **10 µl** of **Pos DNA *S. pneumoniae* C+** to the tube with **PCR-mix-1 *S. pneumoniae/ H. influenzae;***
- add **10 µl** of **Pos DNA *H. influenzae* C+** to the tube with **PCR-mix-1 *S. pneumoniae/ H. influenzae;***
- add **10 µl** of **Pos DNA *N.meningitidis* C+** to the tube with **PCR-mix-1 *N. meningitidis*/ IC*;***
- add **10 µl** of **Pos IC C+** to the tube with **PCR-mix-1 *N. meningitidis*/ IC*;***

Amplification

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

	***Rotor type instruments*[1]**				***Plate type or modular instruments*[2]**			
Stage	***Temp, ℃***	***Time***	***Fluorescence detection***	***Cycle repeats***	***Temp, ℃***	***Time***	***Fluorescence detection***	***Cycle repeats***
Hold	95	15 min	–	1	95	15 min	–	1
Cycling	95	10 s	–	45	95	10 s	–	45
	56	20 s	FAM(Green), JOE(Yellow)		56	30 s	FAM, JOE/HEX/Cy3	
	72	10 s	–		72	10 s	–	

[1] For example Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen)

[2] For example, SaCycler-96™ (Sacace), CFX/iQ5™ (BioRad); Mx3005P™ (Agilent), ABI® 7300/7500/StepOne Real Time PCR (Applied Biosystems), SmartCycler® (Cepheid), LineGeneK® (Bioer)

INSTRUMENT SETTINGS

Rotor-type instruments

Channel	Calibrate/Gain Optimisation…	Threshold	More Settings/ Outlier Removal	Slope Correct
FAM/Green	*from 5 Fl to 10 Fl*	*0.05*	*10 %*	*On*
JOE/Yellow	*from 4 Fl to 8 Fl*	*0.05*	*5-10 %*	*On*

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.

RESULTS ANALYSIS

The results are interpreted by the device software through the presence of crossing of fluorescence curve with the threshold line. Put the threshold line at such level where curves of fluorescence are linear.

- ***Streptococcus pneumoniae*** is detected on the FAM (Green) channel and ***Haemophilus influenzae*** on the JOE (Yellow)/HEX/Cy3 channel with PCR-mix-1 *S. pneumoniae/ H. influenzae;*
- ***Internal Control (IC)*** is detected on the FAM (Green) channel and ***Neisseria meningitidis*** on the JOE (Yellow)/HEX/Cy3 channel with PCR-mix-1 *N. meningitidis*/ IC;

The sample is considered to be positive if the value of **Ct** is different from zero (Ct < Boundary value)

The sample is considered to be negative if the result is positive only on the channel Fam with PCR-mix-1 *N. meningitidis*/ IC and the Ct value is lower than boundary value.

Ct boundary values

Sample	Channel	Boundary Ct (Rotor-type)	Boundary Ct (Plate-type)
CS+	FAM/Green	38	40
C+$_{N.meningitidis}$	JOE/Yellow	38	40
C+$_{S.pneumoniae}$	FAM/Green	38	40
C+$_{H.influenzae}$	JOE/Yellow	38	40
C-	FAM/Green	38	40
Clinical samples	FAM/Green	38	40
	JOE/Yellow	38	40

PERFORMANCE

Sensitivity

Clinical material	DNA extraction kit	PCR kit	Pathogen	Analytical sensitivity, GE/ml*
Cerebrospinal fluid	DNA/RNA-Prep	NHS Meningitidis Real-TM	*Neisseria meningitidis*	$1x10^3$
			Haemophilus influenzae	
			Streptococcus pneumoniae	

* *Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample.*

Specificity

The analytical specificity of **NHS Meningitidis Real-TM** PCR kit is ensured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis.

Specificity was evaluated by testing the following microorganism sand strains: *Enterobacter aerogenes and E. cloacae; Enterococcus faecalis* (GISK 29212); *Escherichia coli* (NCTC 9001) and *E. coli* (ATCC 25922); *Haemophilus parainfluenzae and H. haemolyticus; Klebsiella oxytoca and K. pneumoniae; Listeria monocytogenes; Moraxella catarrhalis; Neisseria cinereae, N. elongate, N. flavescens, N. gonorrhoeae, N. mucosa; N. sicca and N. subflava; Pantoea agglomerans; Proteus mirabilis; Pseudomonas aeruginosa* (ATCC 27853); *Salmonella enteritidis* (GISK 1137) and *S. typhi* (Central Public Health Laboratory (London) 5715); *Shigella flexneri* 2a (GISK 1270) and *S. sonnei* (GISK 9090); *Staphylococcus aureus* (ATCC 25923) and *S. saprophyticus* (ATCC 15305), *S. pneumoniae, S. agalactiae, S. milleri, S. mitis, S. mutans, S. pyogenes, S. salivarius, S. sanguis, S. suis and S. viridians; and Yersinia enterocolitica and Y. pseudotuberculosis*. The analytical specificity was also confirmed by testing human DNA. Non-specific results were not detected.

TROUBLESHOOTING

1. Weak or absent signal of the IC (Fam (Green) channel) with PCR-mix-1 *N. meningitidis*/ IC: retesting of the sample is required.
    - The PCR was inhibited.
        - ⇒ Make sure that you use a recommended DNA extraction method and follow the manufacturer's instructions.
        - ⇒ Re-centrifuge all the tubes before pipetting 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
    - The PCR conditions didn't comply with the instructions.
        - ⇒ Check the PCR conditions and for the IC detection select 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 (Ct > boundary value) sample signal on the Fam/Joe (Yellow)/Cy3/HEX channel: retesting of the sample is required.
3. Joe (Yellow)/Cy3/HEX signal with Negative Control of extraction.
    - Contamination during DNA extraction procedure. All sample results are invalid.
        - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol.
        - ⇒ Use only filter tips during the extraction procedure. Change tips among tubes.
        - ⇒ Repeat the RNA extraction with the new set of reagents.
4. Any signal with Negative PCR Control.
    - Contamination during PCR preparation procedure. All sample results are invalid.
        - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents.
        - ⇒ Pipette the Positive controls at the end.
        - ⇒ 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	**NCE**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control

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