Sacace BIOTECHNOLOGIES

IVD For *in Vitro* Diagnostic Use

CE

NHS Meningitis Real-TM

Handbook

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

REF B25-50FRT

REF TB25-50FRT

Σ 50

NAME

NHS Meningitis Real-TM

INTENDED USE

Kit **NHS Meningitis 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 Meningitis Real-TM Test is based on three major processes: isolation of DNA from specimens, Real Time amplification of the DNA and *NHS Meningitis* 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 Meningitis 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

Module No.1: Real Time PCR kit (B25-50FRT)

Part N° 2 – **"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;

Part N° 3 – **"NHS Meningitis Real-TM":** RealTime 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*

Module No.2: Complete Real Time PCR test with DNA purification kit (TB25-50FRT)

Part N° 1 – **"DNA-Sorb-B":** Sample preparation

- **Lysis Solution**, 15 ml;
- **Washing Solution 1**, 15 ml;
- **Washing Solution 2**, 50 ml;
- **Sorbent**, 1,25 ml;
- **DNA-eluent**, 5,0 ml.

Contains reagents for 50 extractions

Part N° 2 – **"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;

Part N° 3 – **"NHS Meningitis Real-TM":** RealTime 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 (Module No. 1)
- Biological cabinet
- Desktop microcentrifuge for “eppendorf” type tubes (RCF max. 16,000 x g); Eppendorf 5415D or equivalent
- 60°C ± 2°C dry heat block
- Vortex mixer
- Pipettors (capacity 5-40 µl; 40-200 µl; 200-1000 µl) with aerosol barrier
- Sterile pipette tips with filters
- 1,5 ml polypropylene sterile tubes (Sarstedt, QSP, Eppendorf)
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator, Freezer

Zone 2: RT and amplification:

- Real Time Thermal cycler
- Reaction tubes
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Freezer, refrigerator

STORAGE INSTRUCTIONS

Part N° 1 – **“DNA-Sorb-B”** must be stored at 2-8°C.

Part N° 2 – **“Controls”** must be stored at 2-8°C.

Part N° 3 – **“NHS Meningitis 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 Meningitis 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

The user should always pay attention to the following:

- ⚠ Lysis Solution contains guanidine thiocyanate*. Guanidine thiocyanate is harmful if inhaled, or comes into contact with skin or if swallowed. Contact with acid releases toxic gas. (Xn; R: 20/21/22-36/37/38; S: 36/37/39).
- 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.

**** Only for Module No.2***

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 (EN375). 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 Meningitis Real-TM can analyze DNA extracted with **DNA-Sorb-B** ((Sacace, REF K-1-1/B) 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

The following kits are recommended:

- ⇨ **DNA-Sorb-B** (Sacace, REF K-1-1/B)
- ⇨ **DNA/RNA Prep** (Sacace, REF K-2-9)

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.

SPECIMEN AND REAGENT PREPARATION

1. **Lysis Solution** and **Washing Solution** (in case of their storage at +2-8°C) should be warmed up to 60°C until disappearance of ice crystals.
2. Prepare required quantity of 1.5 ml polypropylene tubes.
3. Add to each tube **300 µl** of **Lysis Solution** and **10 µl** of **IC**.
4. Add **100 µl** of **Samples** to the appropriate tube.
5. Prepare Controls as follows:
   - add **100 µl** of **C–** (**Negative Control**) to labeled C*neg*.
6. Vortex the tubes, incubate 5 min at 65°C and centrifuge for 5 sec.
7. Vortex vigorously **Sorbent** and add **25 µl** to each tube.
8. Vortex for 5-7 sec and incubate all tubes for 10 min at room temperature. Vortex periodically
9. Centrifuge all tubes for 1 min at 5000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
10. Add **300 µl** of **Washing Solution 1** to each tube. Vortex vigorously and centrifuge for 1 min at 5000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
11. Add **500 µl** of **Washing Solution 2** to each tube. Vortex vigorously and centrifuge for 1 min at 10000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
12. Repeat step 11.
13. Incubate all tubes with open cap for 5 min at 65°C.
16. Resuspend the pellet in **50 µl** of **DNA-eluent.** Incubate for 5 min at 65°C and vortex periodically.
17. Centrifuge the tubes for 2 min at maximum speed (12000-16000 g). The supernatant contains DNA ready for amplification. The amplification can be performed on the same day of extraction.

PROTOCOL:

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 the reaction mix for required number of samples.

3 For N reactions mix for each PCR-Mix-1 in a new tube:

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

5.0*(N+1) µl of PCR-mix-2

0.5*(N+1) µl of TaqF Polymerase

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

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

N.B. If the DNA-Sorb isolation kit is used as a DNA extraction kit, re-centrifuge all the tubes with extracted DNA for 2 min at maximum speed (12000-16000 g) and take carefully supernatant. N.B. don't disturb the pellet, sorbent inhibit reaction

6 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, °C***	***Time***	***Fluorescence detection***	***Cycle repeats***	***Temp,°C***	***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*	*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 < 40)

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 40.

Ct boundary values

Sample	Channel	Ct
CS+	FAM/Green	38
$C+_{N.meningitidis}$	JOE/Yellow	38
$C+_{S.pneumoniae}$	FAM/Green	38
$C+_{H.influenzae}$	JOE/Yellow	38
C-	FAM/Green	38
Clinical samples	FAM/Green	38
	JOE/Yellow	38

SPECIFICATIONS

Sensitivity

Clinical material	DNA extraction kit	PCR kit	Pathogen	Analytical sensitivity, GE/ml*
Cerebrospinal fluid	DNA/RNA-Prep	NHS Meningitis 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 Meningitis 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 > 40) 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 samples 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 samples 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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