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For Professional Use Only

AmpliSens® *N.meningitidis / H.influenzae / S.pneumoniae*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® ***N.meningitidis / H.influenzae / S.pneumoniae*****-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Neisseria meningitidis*, *Haemophilus influenzae,* and *Streptococcus pneumoniae* DNA in clinical materials (cerebrospinal fluid) by using real-time hybridization-fluorescence detection.

The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Neisseria meningitidis, *Haemophilus influenzae,* and *Streptococcus pneumoniae* detection by the polymerase chain reaction (PCR) is based on the multiplex amplification of the pathogen genome specific region in two tubes using specific primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens®** ***N.meningitidis / H.influenzae / S.pneumoniae*****-FRT** PCR kit is a qualitative test that contains the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. **AmpliSens®** ***N.meningitidis / H.influenzae / S.pneumoniae*****-FRT** PCR kit uses “hot-start,” which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® ***N.meningitidis / H.influenzae / S.pneumoniae*****-FRT** PCR kit is produced in 1 form:

AmpliSens® *N.meningitidis / H.influenzae / S.pneumoniae*-FRT PCR kit variant FRT-50 F, **REF** R-B25(RG,iQ)-CE.

AmpliSens® *N.meningitidis / H.influenzae / S.pneumoniae*-FRT PCR kit variant FRT-50 F includes:

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI	colorless clear liquid	0.6	1 tube
PCR-mix-1-FEP/FRT *Streptococcus pneumoniae* / *Haemophilus influenzae*	colorless clear liquid	0.6	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
DNA-buffer	colorless clear liquid	0.5	1 tube
Positive Control DNA *Neisseria meningitidis*-Flu (C+$_{N.meningitidis}$)	colorless clear liquid	0.1	1 tube
Positive Control DNA *Haemophilus influenzae* (C+$_{H.influenzae}$)	colorless clear liquid	0.1	1 tube
Positive Control DNA *Streptococcus pneumoniae* (C+$_{S.pneumoniae}$)	colorless clear liquid	0.1	1 tube
Positive Control STI-88 (CS+)	colorless clear liquid	0.1	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube

* must be used in the extraction procedure as Negative control of extraction.

** add Internal Control during the DNA extraction procedure directly to the sample/lysis mixture (see the “DNA-sorb-B” **REF** K1-2-50-CE, “RIBO-sorb” **REF** K2-1-Et-50-CE, and “RIBO-prep” **REF** K2-9-Et-50-CE protocols).

AmpliSens® *N.meningitidis / H.influenzae / S.pneumoniae* FRT PCR kit is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- 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.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett

Research, Australia); iCycler iQ or iQ5 (Bio-Rad, USA) or equivalent).

- Disposable polypropylene microtubes for PCR (0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 samples and unused reagents in compliance with local authorities' requirements.
- Samples 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 another suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact, immediately flush with water, seek medical attention.
- 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.

6. SAMPLING AND HANDLING

Obtaining samples of biological materials for PCR-analysis, transportation, and storage are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *N.meningitidis / H.influenzae / S.pneumoniae*-FRT PCR kit is intended for the analysis of DNA extracted with RNA/DNA extraction kits from

- *cerebrospinal fluid*

No less than 1.0 ml of cerebrospinal fluid should be collected for the test to a 2.0-ml disposable tube. Samples can be stored at room temperature for 6 hours, at 2-8 °C for 1 day, at or below minus 16°C for 1 month, and at or below minus 68°C for a long time.

Only one freeze-thaw cycle of clinical material is allowed.

7. WORKING CONDITIONS

AmpliSens® *N.meningitidis / H.influenzae / S.pneumoniae*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits:

- DNA-sorb-B **REF** K1-2-50-CE;
- RIBO-sorb **REF** K2-1-Et-50-CE;
- RIBO-prep **REF** K2-9-Et-50-CE;
- Other nucleic acid extraction kits recommended by FBIS CRIE.

It is recommended to use RIBO-sorb and RIBO-prep extraction kits for clinical samples that are simultaneously tested for *enterovirus* infections.

Extract DNA according to the manufacturer's instructions.

8.3. Preparing PCR

8.3.1. Preparing tubes for PCR

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

Mix the reaction mixture components just before use. Prepare the reaction mixture for the required number of reactions (including clinical and control samples) as specified in

Appendix 1. Carry out all control amplification reactions (positive, negative, and two background with each PCR-mix-1) for testing even one clinical or control sample. Prepare the reagent mixture for an even number of reactions to attain more precise dispensing.

1. Thaw the reagents, vortex the tubes thoroughly, and make sure that there are no drops on the walls of the tubes.
2. Prepare the required number of tubes for amplification of DNA from clinical and control samples.
3. Mix one of the PCR-mixes-1 (**PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI** or **PCR-mix-1-FEP/FRT *Streptococcus pneumoniae* / *Haemophilus influenzae)***, **PCR-mix-2-FRT,** and **polymerase (TaqF)** according to Appendix 1. Vortex the tubes thoroughly. Make sure that there are no drops on the walls of the tubes.
4. Transfer **15 µl** of the prepared mixture to the prepared tubes. Dispose of the unused reaction mixture.
5. Add **10 µl** of **DNA** obtained from clinical or control samples at the extraction stage into the prepared tubes using tips with aerosol barrier.

Avoid transferring sorbent together with the DNA sample in case of extraction by "RIBO-sorb" or "DNA-sorb-B" kits.

6. Carry out the control amplification reactions:

NCA - Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+*N.meningitidis* - Add **10 µl** of **Positive Control DNA *Neisseria meningitidis*-Flu** (for **PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI**) to the tube labeled C+*N.meningitidis* (Positive Control of Amplification).

CS+ - Add **10 µl** of **Positive Control STI-88** (for **PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI**) to the tube labeled CS+ (Positive Control of Amplification).

C+*S.pneumoniae* - Add **10 µl** of **Positive Control DNA *Streptococcus pneumoniae*** (for **PCR-mix-1-FEP/FRT *Streptococcus pneumoniae* / *Haemophilus influenzae***) to the tube labeled C+*S.pneumoniae* (Positive Control of Amplification).

C+*H.influenzae* - Add **10 µl** of **Positive Control DNA *Haemophilus influenzae*** (for **PCR-mix-1-FEP/FRT *Streptococcus pneumoniae* / *Haemophilus influenzae***) to the tube labeled C+*H.influenzae* (Positive Control of Amplification).

8.3.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual.

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

Table 1

Amplification program

	Rotor-type Instruments[1]			Plate-type Instruments[2]		
Step	***Temperature, °C***	***Time***	**Cycles**	***Temperature, °C***	***Time***	**Cycles**
Hold	**95**	15 min	1	**95**	15 min	1
Cycling	**95**	10 s	45	**95**	10 s	45
	56	20 s *fluorescent signal detection*		**56**	25 s *fluorescent signal detection*	
	72	10 s		**72**	10 s	

Fluorescent signal is detected in the channels designed for the FAM/Green and JOE/Yellow/HEX fluorophores on the 2nd step of stage Cycling.

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin.*
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Result interpretation

The results are interpreted by the software of instrument by the crossing (or not-crossing) of the fluorescence curve with the threshold line and shown as the presence (or absence) of Ct (threshold cycle) in the result grid.

Table 2

Correspondence between detection channels and pathogens

Detection channel	**PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI**	**PCR-mix-1-FEP/FRT *Streptococcus pneumoniae / Haemophilus influenzae***
FAM/Green	Internal Control-FL DNA	*Streptococcus pneumoniae* DNA
JOE/Yellow/HEX	*Neisseria meningitidis* DNA	*Haemophilus influenzae* DNA

Results should be interpreted in accordance with Table 3, Important Product Information Bulletin, and Guidelines.

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.
[2] For example, iCycler, iQ5, Mx3000P, Mx3000, DT-96 or equivalent.

Table 3

Result interpretation

PCR-mix-1	Ct value in the channel		Result
	FAM/Green	**JOE/Yellow/HEX**	
PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI	**< boundary value**	> boundary value	*Neisseria meningitidis DNA is not detected*
	> boundary value or < boundary value	**< boundary value**	*Neisseria meningitidis DNA is detected*
	> boundary value	> boundary value	Invalid result Repeat extraction and PCR
PCR-mix-1-FEP/FRT *Streptococcus pneumoniae* / *Haemophilus influenzae*	**< boundary value**	> boundary value	*Streptococcus pneumoniae DNA is detected*
	> boundary value	**< boundary value**	*Haemophilus influenzae DNA is detected*
	> boundary value	> boundary value	*Streptococcus pneumoniae* and *Haemophilus influenzae*[3] *DNA are not detected*

* For boundary values, see the *Important product information bulletin*.

Result of the analysis is considered reliable only if the results for Positive and Negative Controls of amplification as well as Negative Control of extraction are correct (Table 4).

[3] If the Ct value detected in the FAM channel is less than the boundary value (with the use of **PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI**).

Table 4

Results for controls

PCR-mix-1	Control	Stage for control	Ct value in the channel	
			FAM/Green	JOE/Yellow/HEX
PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI	C-	DNA extraction	**< boundary value**	> boundary value
	NCA	PCR	> boundary value	> boundary value
	C+$_{N.meningitidis}$	PCR	> boundary value	**< boundary value**
	CS+	PCR	**< boundary value**	> boundary value
PCR-mix-1-FEP/FRT *Streptococcus pneumoniae* / *Haemophilus influenzae*	C-	DNA extraction	> boundary value	> boundary value
	NCA	PCR	> boundary value	> boundary value
	C+$_{S.pneumoniae}$	PCR	**< boundary value**	> boundary value
	C+$_{H.influenzae}$	PCR	> boundary value	**< boundary value**

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

- If Ct value of the Positive Controls of PCR (C+) is greater than boundary value in the FAM/Green or JOE/Yellow/HEX channels the PCR and detection should be repeated for all samples in which Ct value in the FAM/Green or JOE/Yellow/HEX channels is greater than boundary value with appropriate PCR-mix-1.
- If Ct value of the Negative Control of extraction (C-) (except for PCR-mix-1-FEP/FRT Neisseria meningitidis / STI in the FAM/Green channel) and/or Negative Control of amplification (NCA) (in all channels) is less than boundary value, analysis should be repeated (starting from DNA extraction) for all samples in which target pathogen DNA of was detected.
- If no signal is detected for the positive controls of amplification, it may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components did not comply with the manufacturer's instruction, or that the reagent kit expired. Programming of the used instrument, storage conditions, and the expiration

date of the reagents should be checked, and then PCR should be repeated.

- If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample that has a fluorescence curve without the typical exponential growth phase (the curve is linear), this may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be repeated (if iCycler iQ or iQ5 instruments are used).

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® ***N.meningitidis / H.influenzae / S.pneumoniae*-FRT** PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the AmpliSens® *N.meningitidis / H.influenzae / S.pneumoniae*-FRT PCR kit (except for PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI, PCR-mix-1-FEP/FRT *Streptococcus pneumoniae / Haemophilus influenzae,* PCR-mix-2-FRT, and polymerase (TaqF)) are to be stored at 2–8 °C when not in use. All components of the AmpliSens® *N.meningitidis / H.influenzae / S.pneumoniae*-FRT PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI, PCR-mix-1-FEP/FRT *Streptococcus pneumoniae / Haemophilus influenzae,* PCR-mix-2-FRT, and Polymerase (TaqF) are to be stored at temperature from minus 24 to minus 16 °C when not in use.

PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI and PCR-mix-1-FEP/FRT *Streptococcus pneumoniae / Haemophilus influenzae* are to be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

Clinical material	DNA extraction kit	PCR kit	Pathogen	Analytical sensitivity, GE/ml*
Cerebrospinal fluid	RIBO-prep	PCR kit variant FRT-50 F	*Neisseria meningitidis*	$1x10^3$
			Haemophilus influenzae	
			Streptococcus pneumoniae	

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

13.2. Specificity

The analytical specificity of **AmpliSens® *N.meningitidis / H.influenzae / S.pneumoniae*-FRT** 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. pneumonia**e**; 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.

The clinical specificity of **AmpliSens® *N.meningitidis / H.influenzae / S.pneumoniae*-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *N.meningitidis / H.influenzae / S.pneumoniae*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+*N.meningitidis* C+*H.influenzae* C+*S.pneumoniae***	Positive controls of amplification
FBIS CRIE	Federal Budget Institute of Science "Central Research Institute for Epidemiology"	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
27.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”