

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



For Professional Use Only

## Instruction Manual

### KEY TO SYMBOLS USED

	Catalogue number		Caution
	Batch code		Sufficient for
	<i>In vitro</i> diagnostic medical device		Use-by Date
	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer		Negative control of amplification
	Date of manufacture		Negative control of extraction
	Authorized representative in the European Community		Positive controls of amplification
			Internal control

### 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 material (cerebrospinal fluid) using real-time hybridization-fluorescence detection of amplified products.

**NOTE:** 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 using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and dUTP. The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because dUTP is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR. The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE
Name of PCR-mix-1-FEP/FRT	DNA-target	
<i>Neisseria meningitidis</i> / STI	Internal Control-FL (IC) DNA	<i>Neisseria meningitidis</i> DNA
<i>Streptococcus pneumoniae</i> / <i>Haemophilus influenzae</i>	<i>Streptococcus pneumoniae</i> DNA	<i>Haemophilus influenzae</i> DNA
Name of PCR-mix-1-FEP/FRT	Target gene	
<i>Neisseria meningitidis</i> / STI	Artificially synthesized sequence	ctr A
<i>Streptococcus pneumoniae</i> / <i>Haemophilus influenzae</i>	ply	bex A

### 3. CONTENT

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

variant FRT-50 F, R-B25(RG,iQ)-CE.

Variant FRT-50 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FEP/FRT <i>Neisseria meningitidis</i> / STI	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-mix-1-FEP/FRT <i>Streptococcus pneumoniae</i> / <i>Haemophilus influenzae</i>	clear liquid from colorless to light lilac colour	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 <i>Neisseria meningitidis</i> -Flu (C+N.meningitidis)	colorless clear liquid	0.1	1 tube
Positive Control DNA <i>Haemophilus influenzae</i> (C+H.influenzae)	colorless clear liquid	0.1	1 tube
Positive Control DNA <i>Streptococcus pneumoniae</i> (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 10 µl of Internal Control-FL (IC) during the DNA extraction procedure directly to the sample/lysis mixture (see the DNA-sorb-B K1-2-50-CE, RIBO-sorb K2-1-Et-50-CE or RIBO-prep K2-9-Et-50-CE protocols).

Variant FRT-50 F 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 a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany), iCycler iQ or iQ5 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml) when working with PCR kit variant FRT-100 F:
  - a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  - b) 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

### 5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. 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 regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where 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

**NOTE:** 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 analysis of the DNA extracted with DNA extraction kits from the clinical material (cerebrospinal fluid).

Obtain cerebrospinal fluid (CSF) using disposable needles and collect it in a amount of not less than 1.0 ml into the disposable dry 2.0-ml plastic tubes. Tightly close the tubes, avoiding clearance and crumpling of the tube caps. Mark the tubes.

Samples can be stored at the room temperature for 6 hours, at 2-8 °C for 1 day, at the temperature below minus 16°C for 1 month, and at the temperature minus 68°C for a long time.

**NOTE:** Only one freeze-thaw cycle of the 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;

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

The DNA extraction of each test sample is carried out in the presence of **Internal Control-FL (IC)**.

**NOTE:** Extract DNA according to the manufacturer's protocol.

### 8.2. Preparing PCR

#### 8.2.1. Preparing tubes for PCR

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

Reaction mixture components should be mixed just before analysis with calculating for the required number of reactions (test and control samples) according to the Table 2. Note that even for analysis of one test DNA sample, it is necessary to carry out all controls of the PCR stage: Positive Control of Amplification (C+) and Negative Control of Amplification (NCA) for each PCR-mix.

**NOTE:** It is recommended to mix the reagents for an even reaction number to ensure more exact dosage

1. Thaw all the reagents, vortex the tubes thoroughly, and sediment drops from walls of tubes.
2. Take the required number of tubes for amplification of the DNA from clinical and control samples.
3. To prepare the reaction mixture, 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 Table 2. Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

Table 2

Scheme of reaction mixture preparation			
Reagent volume per one reaction (µl)	Reagent volume for the specified number of reactions (µl)		
	10.00	5.00	0.50
Number of reactions <sup>1</sup>	PCR-mix-1-FEP/FRT	PCR-mix-2-FRT	Polymerase (TaqF)
6	60	30	3.0
8	80	40	4.0
10	100	50	5.0
12	120	60	6.0
14	140	70	7.0
16	160	80	8.0
18	180	90	9.0
20	200	100	10.0
22	220	110	11.0
24	240	120	12.0
26	260	130	13.0
28	280	140	14.0
30	300	150	15.0
32	320	160	16.0

<sup>1</sup> Number of test samples including controls of extraction (N), controls of amplification and one extra reaction (N+3+1).

4. Transfer 15 µl of the prepared mixture to each tube. Dispose of the unused reaction mixture.
5. Using tips with aerosol filter, add 10 µl of DNA obtained at the DNA extraction stage.

**NOTE:** Avoid transferring sorbent together with the DNA sample extracted 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.men** – 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.pne** – 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.influ** – 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).
- C–** – Add 10 µl of the sample extracted from the **Negative Control reagent** to the tube labeled **C–** (Negative control of Extraction)

### 8.2.2. Amplification

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

Table 3

Step	Rotor-type instruments <sup>2</sup>			Plate-type instruments <sup>3</sup>		
	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		56	25 s	
		Fluorescence acquiring			Fluorescence acquiring	
	72	10 s		72	10 s	

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores (other channels are enabled if several tests are simultaneously carried out in a single run).

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and *Guidelines* [2].
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

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels (for the FAM and JOE fluorophores).

Table 4

Correspondence between detection channels and pathogens		
Channel for the fluorophore	PCR-mix-1-FEP/FRT <i>Neisseria meningitidis</i> / STI	PCR-mix-1-FEP/FRT <i>Streptococcus pneumoniae</i> / <i>Haemophilus influenzae</i>
FAM	Internal Control-FL DNA	<i>Streptococcus pneumoniae</i> DNA
JOE	<i>Neisseria meningitidis</i> DNA	<i>Haemophilus influenzae</i> DNA

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a Ct value for the DNA sample in the corresponding column of the results grid. Results should be interpreted in accordance with Table 5, *Important Product Information Bulletin* and *Guidelines* [2].

Table 5

Result interpretation			
PCR-mix-1	Ct value in the channel for fluorophore		Result
	FAM	JOE	
PCR-mix-1-FEP/FRT <i>Neisseria meningitidis</i> / STI	< boundary value	> boundary value	<i>Neisseria meningitidis</i> DNA is not detected
	> boundary value or < boundary value	< boundary value	<i>Neisseria meningitidis</i> DNA is detected
	> boundary value	> boundary value	<b>Invalid result</b> Repeat extraction and PCR
	< boundary value	> boundary value	<i>Streptococcus pneumoniae</i> DNA is detected
PCR-mix-1-FEP/FRT <i>Streptococcus pneumoniae</i> / <i>Haemophilus influenzae</i>	> boundary value	< boundary value	<i>Haemophilus influenzae</i> DNA is detected
	> boundary value	> boundary value	<i>Streptococcus pneumoniae</i> and <i>Haemophilus influenzae</i> <sup>4</sup> DNA are not detected
	< boundary value	> boundary value	<i>Streptococcus pneumoniae</i> DNA is detected

**NOTE:** Boundary Ct values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also *Guidelines* [2]

The result of the 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 (see Table 6).

<sup>2</sup> For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.

<sup>3</sup> For example, iCycler, IQ5, Mx3000P, Mx3000, DT-96 or equivalent.

<sup>4</sup> If the Ct value detected in the channel for FAM fluorophore is less than the boundary value (with the use of **PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI**).

Table 6

## Results for controls

PCR-mix-1	Control	Stage for control	Ct value in the channel for fluorophore	
			FAM	JOE
PCR-mix-1-FEP/FRT <i>Neisseria meningitidis</i> / STI	C-	DNA extraction	< boundary value	> boundary value
	NCA	PCR	> boundary value	> boundary value
	C+ <i>N.meningitidis</i>	PCR	> boundary value	< boundary value
	CS+	PCR	< boundary value	> boundary value
PCR-mix-1-FEP/FRT <i>Streptococcus pneumoniae</i> / <i>Haemophilus influenzae</i>	C-	DNA extraction	> boundary value	> boundary value
	NCA	PCR	> boundary value	> boundary value
	C+ <i>S.pneumoniae</i>	PCR	< boundary value	> boundary value
	C+ <i>H.influenzae</i>	PCR	> boundary value	< boundary value

## 10. TROUBLESHOOTING

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

- If the Ct value determined for the Positive Controls of Amplification (C+) in the channels for the FAM or JOE fluorophores is greater than the boundary Ct value, the amplification and detection should be repeated for all samples in which Ct value determined in the channels for the FAM or JOE fluorophores was greater than the boundary Ct value for required PCR-mix-1.
- If the Ct value determined for the Negative Control of Extraction (C-) (except for PCR-mix-1-FEP/FRT *Neisseria meningitidis* / STI in the channel for FAM fluorophore) and/or Negative Control of Amplification (NCA) (in all channels) is less than the boundary Ct value, PCR-analysis should be repeated (beginning with DNA extraction) for all samples in which target pathogen DNA was detected.

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 are to be stored at 2–8 °C when not in use (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)). All components of the AmpliSens® *N.meningitidis* / *H.influenzae* / *S.pneumoniae*-FRT PCR kit are stable until the expiry date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

**NOTE:** 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 the temperature from minus 24 to minus 16 °C when not in use.

**NOTE:** 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	Nucleic acid extraction kit	Pathogen	Analytical sensitivity, GE/ml <sup>5</sup>
Cerebrospinal fluid	RIBO-prep	<i>Neisseria meningitidis</i>	1x10 <sup>3</sup>
		<i>Haemophilus influenzae</i>	
		<i>Streptococcus pneumoniae</i>	

## 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.

The specificity was proved on the following strains of microorganisms: *Enterobacter aerogenes*; *Enterobacter cloacae*; *Enterococcus faecalis* (GISK 29212); *Escherichia coli* (NCTC 9001); *Escherichia coli* (ATCC 25922); *Haemophilus parainfluenzae*; *Haemophilus Haemolyticus*; *Klebsiella oxytoca*; *Klebsiella pneumoniae*; *Listeria monocytogenes*; *Moraxella catarrhalis*; *Neisseria cinerea*; *Neisseria elongate*; *Neisseria flavescens*; *Neisseria gonorrhoeae*; *Neisseria mucosa*; *Neisseria sicca*; *Neisseria subflava*; *Pantoea agglomerans*; *Proteus mirabilis*; *Pseudomonas aeruginosa* (ATCC 27853); *Salmonella enteritidis* (GISK 1137); *Salmonella typhi* (Central Public Health Laboratory (London) 5715); *Shigella flexneri* 2a (GISK 1270); *Shigella sonnei* (GISK 9090); *Staphylococcus aureus* (ATCC 25923); *Staphylococcus saprophyticus* (ATCC 15305); *Streptococcus pneumoniae*; *Streptococcus agalactiae*; *Streptococcus milleri*; *Streptococcus mitis*; *Streptococcus mutans*; *Streptococcus pyogenes*; *Streptococcus salivarius*; *Streptococcus sanguis*; *Streptococcus suis*; *Streptococcus viridians*; *Yersinia enterocolitica*; *Yersinia 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

- 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.
- Guidelines to the AmpliSens® *N.meningitidis* / *H.influenzae* / *S.pneumoniae*-FRT PCR kit for qualitative detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* DNA in the clinical material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology".

## 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.

## 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"
20.08.15 PM	Text	Corrections according to the template.
	8.2.1. Preparing tubes for PCR	Scheme of reaction mixture preparation was added from Appendix 1
	9. Data analysis	The sections were rewritten
	10. Troubleshooting	
14. References	The reference to the Guidelines was added	
15.12.17 PM	3. Content	The color of reagents were specified
28.01.19 DV	2. Principle of PCR detection	The information about the enzyme UDG was added. The information about "hot-start" was corrected
	Through the text	The text formatting was changed
08.05.20 MA	Footer	The phrase "Not for use in the Russian Federation" was added
	2. Principle of PCR detection	The table with targets was added
17.03.21 MA	–	The name, address and contact information for Authorized representative in the European Community was changed

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<sup>5</sup> Genome equivalents (GE) of the pathogen agent per 1 ml of a sample.