AmpliSens® *Streptococcus pyogenes*-screen/monitor-FRT PCR kit
Instruction Manual

AmpliSens®

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1. INTENDED USE

-AmpliSens® *Streptococcus pyogenes*-screen/monitor-FRT PCR kit PCR kit is an *in vitro* nucleic acid amplification test for quantitative detection of *Streptococcus pyogenes* DNA in the biological material (oropharyngeal swabs, whole blood, tissue (biopsy, surgical, autopsy) material, synovial fluid, discharge of erosive and ulcerative lesions of the skin, cerebrospinal fluid (CSF), urine) taken from the persons suspected of streptococcal infection without distinction of form and presence of manifestation, using real-time hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

The principle of testing is based on the DNA extraction from test samples together with the exogenous internal control (Internal Control-FL (IC)) and simultaneous amplification of DNA fragments of the detected microorganism and DNA of the internal control with hybridization-fluorescence detection. Exogenous internal control (Internal Control-FL (IC)) allows to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition.

*Streptococcus pyogenes* detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. In the real-time PCR, the amplified product is detected with the use of 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.

The quantitative analysis of *Streptococcus pyogenes* DNA is based on the linear dependence between the initial concentration of DNA target in a test sample and the cycle threshold (*Ct*) (the cycle of beginning of fluorescence signal exponential growth). For the quantitative analysis amplification of DNA from the test samples is carried out simultaneously with DNA-calibrators (samples with the known concentration of the DNA target). Based on the amplification results of DNA-calibrators a calibration line is plotted and it is used for the estimation of concentration of the DNA target in the test samples.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glicosylase (UDG) and deoxyuridine triphosphate. 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 deoxyuridine triphosphate 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.

At the amplification stage 2 reactions are carried out in one tube simultaneously: amplification of *Streptococcus pyogenes* DNA as well as amplification of Internal Control-FL (IC) DNA. The results of amplification of *Streptococcus pyogenes* DNA and Internal Control-FL (IC) DNA are registered in 2 different fluorescence channels.

<table>
<thead>
<tr>
<th>Channel</th>
<th>FAM</th>
<th>JOE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA-target</td>
<td>Internal Control-FL (IC) DNA</td>
<td><em>Streptococcus pyogenes</em> DNA</td>
</tr>
<tr>
<td>Target gene</td>
<td>Artificially synthesized sequence</td>
<td>erythrogenic toxin B (<em>speB</em>) gene</td>
</tr>
</tbody>
</table>

### 3. CONTENT

**AmpliSens**® *Streptococcus pyogenes*-screen/monitor-FRT PCR kit is produced in 2 forms:

**AmpliSens**® *Streptococcus pyogenes*-screen/monitor-FRT PCR kit variant FRT-100 FN [REF] H-2171-1-1-CE.

**AmpliSens**® *Streptococcus pyogenes*-screen/monitor-FRT PCR kit variant FRT-L [REF] H-2172-1-14-CE.

**AmpliSens**® *Streptococcus pyogenes*-screen/monitor-FRT PCR kit variant FRT-100 FN includes:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
<th>Volume, ml</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-mix-FL <em>Streptococcus pyogenes</em></td>
<td>clear liquid from colorless to light lilac colour</td>
<td>1.2</td>
<td>1 tube</td>
</tr>
<tr>
<td>PCR-bufer-H</td>
<td>colorless clear liquid</td>
<td>0.6</td>
<td>1 tube</td>
</tr>
<tr>
<td>C1 SP</td>
<td>colorless clear liquid</td>
<td>0.2</td>
<td>1 tube</td>
</tr>
<tr>
<td>C2 SP</td>
<td>colorless clear liquid</td>
<td>0.2</td>
<td>1 tube</td>
</tr>
<tr>
<td>TE-bufer</td>
<td>colorless clear liquid</td>
<td>0.2</td>
<td>1 tube</td>
</tr>
<tr>
<td>Internal Control-FL (IC)*</td>
<td>colorless clear liquid</td>
<td>1.0</td>
<td>1 tube</td>
</tr>
<tr>
<td>Negative Control (C-)**</td>
<td>colorless clear liquid</td>
<td>1.2</td>
<td>2 tubes</td>
</tr>
<tr>
<td>Positive Control <em>Streptococcus pyogenes</em>**</td>
<td>colorless clear liquid</td>
<td>0.1</td>
<td>1 tube</td>
</tr>
</tbody>
</table>
* add 10 µl of Internal Control-FL (IC) during the DNA extraction procedure directly to the sample/lysis mixture (see RIBO-prep, REF K2-9-Et-100-CE protocol).

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

*** must be used in the extraction procedure as Positive Control of Extraction.

AmpliSens® Streptococcus pyogenes-screen/monitor-FRT PCR kit variant FRT-100 FN is intended for 110 reactions (including controls).

AmpliSens® Streptococcus pyogenes-screen/monitor-FRT PCR kit variant FRT-L includes:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
<th>Volume, ml</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-mix <em>Streptococcus pyogenes</em>-Lyo</td>
<td>white powder</td>
<td>-</td>
<td>96 tubes of 0.2 ml</td>
</tr>
<tr>
<td>C1 SP</td>
<td>colorless clear liquid</td>
<td>0.5</td>
<td>1 tube</td>
</tr>
<tr>
<td>C2 SP</td>
<td>colorless clear liquid</td>
<td>0.5</td>
<td>1 tube</td>
</tr>
<tr>
<td>TE-buffer</td>
<td>colorless clear liquid</td>
<td>0.5</td>
<td>1 tube</td>
</tr>
<tr>
<td>Internal Control-FL (IC)*</td>
<td>colorless clear liquid</td>
<td>1.0</td>
<td>1 tube</td>
</tr>
<tr>
<td>Negative Control (C-)**</td>
<td>colorless clear liquid</td>
<td>1.2</td>
<td>2 tubes</td>
</tr>
<tr>
<td>Positive Control <em>Streptococcus pyogenes</em> ***</td>
<td>colorless clear liquid</td>
<td>0.1</td>
<td>1 tube</td>
</tr>
</tbody>
</table>

* add 10 µl of Internal Control-FL (IC) during the DNA extraction procedure directly to the sample/lysis mixture (see RIBO-prep, REF K2-9-Et-100-CE protocol).

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

*** must be used in the extraction procedure as Positive Control of Extraction.

AmpliSens® Streptococcus pyogenes-screen/monitor-FRT PCR kit variant FRT-L is intended for 96 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- Transport medium with mucolytic agent
- Transport medium for storage and transportation of respiratory swabs.
- Plastic container (50-60 ml) for sampling, storage and transportation of biological samples.
- Vacuette® blood collection system.
- Urine analysis preservative tube.
- Urine transfer straw.
- Flocked swab for collection, transportation and storage of biological samples
- Reagent for pretreatment of whole peripheral and umbilical blood.
• Sterile tools (individual for each sample) for homogenization (porcelain mortar and mallet) or homogenizer for pretreatment of viscera material.
• Microcentrifuge for Eppendorf tubes (RCF max. 12,000 x g).
• Vortex mixer.
• Vacuum aspirator with flask for removing supernatant.
• DNA extraction kit.
• Disposable powder-free gloves and laboratory coat.
• Pipettes (adjustable).
• Sterile RNase-free and pipette tips with filters (up to 100 µl, 200 µl).
• Tube racks.
• PCR box.
• Real-time instruments with 3 (or more) independent detection channels (for example, Rotor-Gene Q (QIAGEN, Germany), CFX96 (Bio-Rad, USA)).
• Disposable polypropylene PCR tubes:
  a) tightly closed 2.0-ml tubes for sampling;
  b) screwed or tightly closed 1.5-ml tubes for pretreatment and reaction mixture preparation;
  c) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
  d) thin-walled 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 with the range from 2 to 8 °C.
• Deep-freezer with the range 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 (samples, 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 afterward.
• Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work
Do not use the PCR kit if the internal packaging was damaged or its appearance was changed.
Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
Do not use a kit after its expiration date.
Dispose of all samples and unused reagents in accordance with local regulations.
Samples should be considered potentially infectious and handled in a biological cabinet in accordance 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.
While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
Safety Data Sheets (SDS) are available on request.
The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section “Content”).
The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
Use of this product should be limited to personnel trained in the 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 to 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

AmpliSens® *Streptococcus pyogenes*-screen/monitor-FRT PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the biological material (oropharyngeal swabs, whole blood, tissue (biopsy, surgical, autopsy) material, synovial fluid, discharge of erosive and ulcerative lesions of the skin, cerebrospinal fluid (CSF), urine).
Sampling

6.1 Oropharyngeal swabs

The material is taken with a sterile dry probe. Rotate the probe over the tonsillar area, palatine arches, and posterior area of the oropharynx. When the material is obtained, place the working part of the probe into the sterile disposable tube with 500 µl of Transport Medium for Storage and Transportation of Respiratory Swabs (REF 959-CE, REF 957-CE, REF 958-CE). Break off the plastic stick at the distance no more than 0.5 cm from the working part. Close the tube with the working part of the probe in it. Mark the tube.

6.2 Whole blood

Blood should be taken after overnight fasting or in 3 hour after eating by a disposable 0.8-1.1 mm diameter needle into the tube with EDTA (special vacuum system Vacuette® (lavender caps – 6 % EDTA)). After blood sampling the tube should be gently inverted several times for the thoroughly mixing with the anticoagulant. (Otherwise, blood will coagulate and DNA extraction will be impossible!) Place the tube in the tube rack.

6.3 Tissue (biopsy, surgical, autopsy) material

The material should be taken from the proposed pathogen location, from the lesional tissue or the area surrounding the lesional tissue.

The tissue pieces (no more than 5 mm in a diameter) should be placed into the disposable 2.0-ml tubes.

The tissue pieces (more than 5 mm in a diameter) should be placed into the disposable 50-ml containers with wide mouth.

6.4 Synovial fluid

Synovial fluid should be collected in an amount no less than 1 ml using disposable needles into disposable 2.0-ml tubes.

6.5 Discharge of erosive and ulcerative lesions of the skin

Samples are collected rotating dry sterile probe onto the erosive and ulcerative lesions of the skin.

When the material is obtained, place the working part of the probe into the sterile disposable tube with 500 µl of Transport Medium with Mucolytic Agent (REF 952-CE; REF 953-CE). Break off the plastic stick at the distance no more than 0.5 cm from the working part. Close the tube with the working part of the probe in it. Mark the tube.
6.6  *Cerebrospinal fluid (CSF).*

Cerebrospinal fluid is collected in an amount no less than 1 ml by puncturing the lumbar, suboccipital area, or cerebral ventricles using sterile puncture needle into disposable 2.0-ml tubes.

6.7  *Urine.*

The first portion of first void urine is taken for PCR-analysis in an amount no more than 15 ml into the dry sterile container (60 ml).

The above-mentioned samples can be stored before pretreatment/PCR analysis:
- at the temperature from 18 to 25 °C – no more than 8 hours;
- at the temperature from 2 to 8 °C – no more than 2 days;
- at the temperature from minus 24 to minus 16 °C – for 1 year (except for whole blood and urine samples).

Only one freeze-thawing cycle is acceptable.

⚠️ Freezing of whole blood and urine samples is unacceptable!

It is allowed to transport the above-mentioned material at the temperature from 2 to 8 °C for 1 day.

*Pretreatment*

6.8  Pretreatment of oropharyngeal swabs and discharge of erosive and ulcerative lesions of the skin is not required.

6.9  *Whole blood.*

The whole blood samples are to be pretreated. Transfer 1.0 ml of whole blood to the disposable 1.5-ml tube. Centrifuge at 8,000-9,000 g (for example, 12,000-13,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 5 minutes. Remove plasma. Add 1.0 ml of *Hemolytic* ([REF] 137-CE) to the pellet. Gently vortex the tubes and leave them for 15 minutes at room temperature (from 18 to 25°C), stirring occasionally. Centrifuge at 4,000 g (for example, 8,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 5 minutes. Remove the supernatant using vacuum aspirator leaving 100 µl of the pellet. After washing the cell pellet should be white, only a small pinkish bloom on the pellet is allowed (the remains of the destroyed erythrocytes). The washing using *Hemolytic* ([REF] 137-CE) may be repeated if necessary. The obtained pellet must be immediately lysed (in case of extraction using RIBO-prep add 300 µl of Solution for Lysis and then extract DNA in accordance with the *Instruction Manual* enclosed to the RIBO-prep reagent kit without adding Solution for Lysis once again).
The pretreated samples of whole blood can be stored before the PCR-analysis:
– at the temperature from 18 to 25 °C – for no more than 6 hours;
– at the temperature from minus 24 to minus 16 °C – for 1 year;
– at the temperature ≤ –68 °C – for a long time.
Only one freeze-thawing cycle is acceptable.

6.10 Tissue (biopsy, surgical, autopsy) material.
Place the tissue (biopsy, surgical, autopsy) material (5-10 mm in a diameter) to a sterile porcelain mortar and grind it up using a mallet. Add 1 ml of Transport Medium with Mucolytic Agent (REF 952-CE; REF 953-CE) to the obtained homogenate and thoroughly mix using a mallet. Use 100 µl of suspension for DNA extraction.
Place the tissue (biopsy, surgical, autopsy) material (less than 5 mm in a diameter) to a sterile porcelain mortar and grind it up using a mallet. Add 0.5 ml of Transport Medium with Mucolytic Agent (REF 952-CE; REF 953-CE) to the obtained homogenate and thoroughly mix using a mallet. Use 100 µl of suspension for DNA extraction.

6.11 Synovial fluid.
Transfer 1.0 ml of synovial fluid to the disposable 1.5-ml tube. Centrifuge at 8,000-9,000 g (for example, 12,000-13,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 5 minutes. Remove the supernatant leaving 100 µl of the pellet for subsequent DNA extraction.

6.12 Cerebrospinal fluid (CSF).
Transfer 1.0 ml of cerebrospinal fluid (CSF) to the disposable 1.5-ml tube. Centrifuge at 8,000-9,000 g (for example, 12,000-13,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 5 minutes. Remove the supernatant leaving 100 µl of the pellet for subsequent DNA extraction.

6.13. The urine samples are to be pretreated.
Transfer 1 ml of urine into the sterile disposable 1.5-ml tube. Centrifuge at 8,000-9,000 g (for example, 12,000-13,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 5 min. Carefully remove the supernatant using the vacuum aspirator and leaving the pellet and 100 µl of supernatant. Add 100 µl of Transport Medium with Mucolytic Agent (REF 952-CE; REF 953-CE) and vortex thoroughly. Extraction should be carried out from 100 µl of the sample.

The pretreated samples of above-mentioned material can be stored before the PCR-analysis:
– at the temperature from 18 to 25 °C – for no more than 6 hours;
– at the temperature from 2 to 8 °C – for no more than 1 day;
- at the temperature from minus 24 to minus 16 °C – for a year;
- at the temperature ≤ −68 °C – for a long time.

Only one freeze-thawing cycle is acceptable.

**Interfering substances and limitations of using test material samples**

The excessive amount of impurities in biological material such as mucus, blood, pus, and others can lead to the amplification reaction inhibition.

The next samples are inapplicable for analysis:
- the samples of biological material collected more than 48 hours before the delivery to the laboratory;
- the whole blood samples, collected in the tubes with heparin as anticoagulant,
- the whole blood samples, containing blood clot or which has been exposed to freezing.
- the freezed urine samples.

To reduce the risk of obtaining a false negative result due to the presence of interfering substances in the sample the Internal Control (Internal Control-FL (IC)) is used in the PCR kit. The Internal Control is added in each biological sample at the extraction stage. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.

7. WORKING CONDITIONS

AmpliSens® *Streptococcus pyogenes*-screen/monitor-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:

- **RIBO-prep.** REF K2-9-Et-100-CE.

  ¡ **If using the RIBO-prep kit** extract the DNA according to the manufacturer’s protocol.

  The volumes of reagents and samples when the DNA is extracted by the RIBO-prep reagent kit:

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

  Add 10 µl of Internal Control-FL (IC) to each tube.

  The volume of the test sample is 100 µl.

  Add 100 µl of Negative Control (C–) into the tube labeled C– (Negative Control of Extraction).

  Add 10 µl of Positive Control *Streptococcus pyogenes* and 90 µl of Negative Control (C–) into the tube labeled PCE (Positive Control of Extraction).

  The volume of elution is 50 µl.
8.2. Preparing PCR
8.2.1. Preparing tubes for PCR
**Variant FRT-100 FN**
The total reaction volume is 25 µl, the volume of the DNA sample is 10 µl.
The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.
1. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:
   - 10 µl of PCR-mix-FL *Streptococcus pyogenes*,
   - 5 µl of PCR-buffer-H.
Prepare the reaction mixture for the total number of test and control samples plus several extra reactions. Number of control samples see in item 7.

![](Warning-icon) Prepare the reaction mixture just before use.

2. Thaw the tubes with PCR-mix-FL *Streptococcus pyogenes* and PCR-buffer-H. Thoroughly vortex the tubes with PCR-mix-FL *Streptococcus pyogenes* and PCR-buffer-H and sediment the drops by vortex.
3. In a new tube prepare the reaction mixture. Mix the required quantities of PCR-mix-FL *Streptococcus pyogenes* and PCR-buffer-H. Sediment the drops by vortex.
4. Take the required number of the tubes or strips taking into account the number of test samples and control samples.
5. Transfer 15 µl of the prepared reaction mixture to each tube. Discard the unused reaction mixture.
6. Add 10 µl of DNA samples extracted from test samples at the DNA extraction stage using tips with filter.
7. Carry out the control amplification reactions:
   - **C1** – Add 10 µl of C1 SP to two tubes with reaction mixture
   - **C2** – Add 10 µl of C2 SP to two tubes with reaction mixture
   - **C-** – Add 10 µl of the sample extracted from the Negative Control (C-) reagent to the tube with reaction mixture
   - **PCE** – Add 10 µl of the sample extracted from Positive Control *Streptococcus pyogenes* to the tube with reaction mixture

![](Warning-icon) It is also necessary to carry out Negative Control of Amplification (NCA) at suspicion on possible contamination

- **NCA** – Add 10 µl of TE-buffer to the tube with reaction mixture
**Variant FRT-L**

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

Use disposable filter tips for adding reagents, DNA and control samples into tubes.

1. Take the required number of the tubes with ready-to-use lyophilized reaction mixture **PCR-mix *Streptococcus pyogenes*-Lyo** for amplification of DNA from test and control samples (the number of control samples see in point 3).

2. Add 25 µl of **DNA samples** extracted from test samples into the prepared tubes.

   - Mix the tubes thoroughly by pipetting avoiding foaming.

3. Carry out the control reactions:
   - **C1** – Add 25 µl of **C1 SP** to two tubes with reaction mixture
   - **C2** – Add 25 µl of **C2 SP** to two tubes with reaction mixture
   - **C-** – Add 25 µl of **the sample extracted from the Negative Control (C-) reagent** to the tube with reaction mixture
   - **PCE** – Add 25 µl of **the sample extracted from Positive Control *Streptococcus pyogenes*** to the tube with reaction mixture

   - It is also necessary to carry out Negative Control of Amplification (NCA) at suspicion on possible contamination

   - **NCA** – Add 25 µl of **TE-buffer** to the tube with reaction mixture

   - Mix the tubes thoroughly by pipetting avoiding foaming.

### 8.2.2. Amplification

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

   **AmpliSens unified amplification program for rotor-¹ and plate-type² instruments**

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature, °C</th>
<th>Time</th>
<th>Fluorescence detection</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>15 min</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>95</td>
<td>15 min</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>95 60</td>
<td>10 s 20 s</td>
<td>FAM, JOE</td>
<td>45</td>
</tr>
</tbody>
</table>

   Any combination of the tests including test with reverse transcription and amplification can be performed in one instrument simultaneously with the use of the unified amplification program. If several tests in “multiprime” format are carried out simultaneously, the detection is enabled in other used channels except for the specified ones. If only the tests for pathogen agent DNA detection are performed in one instrument then the first step of reverse transcription (50 °C – 15 minutes) can be omitted for time saving

¹) For example, Rotor-Gene Q (QIAGEN, Germany) or equivalent.
²) For example, CFX96 (Bio-Rad, USA) or equivalent.
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.

   ! It is recommended to sediment drops from walls of tubes by short centrifugation before placing them into the instrument.

   Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument

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:

<table>
<thead>
<tr>
<th>Channel for the fluorophore</th>
<th>FAM</th>
<th>JOE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal registration, indicating the amplification product accumulation</td>
<td>Internal Control-FL (IC) DNA</td>
<td><em>Streptococcus pyogenes</em> DNA</td>
</tr>
</tbody>
</table>

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 of the DNA sample in the corresponding column of the results grid.

Principle of interpretation is the following

Based on the obtained *Ct* values and specified concentration values of DNA calibrators (C1 and C2) a calibration line is automatically plotted and the values of *Streptococcus pyogenes* DNA GE in 1 ml of tested and control samples are calculated.

! Concentration values of calibrators are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

<table>
<thead>
<tr>
<th>Results interpretation for the test samples</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invalid</td>
<td>The <em>Ct</em> value in the channel for the FAM fluorophore is absent or determined greater than the boundary value. The PCR analysis (beginning with the DNA extraction stage) should be repeated for this sample.</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em> DNA is not detected</td>
<td>The <em>Ct</em> value for <em>Streptococcus pyogenes</em> DNA is absent or determined greater than the boundary value and the <em>Ct</em> value determined in the channel for the FAM fluorophore is less than the boundary value. The result is <em>Streptococcus pyogenes</em> is not detected.</td>
</tr>
<tr>
<td>less than 1x10³ GE/ml</td>
<td><em>Streptococcus pyogenes</em> DNA was detected in concentration less than the linear measurement range of the PCR kit. The result is less than 1x10³ <em>Streptococcus pyogenes</em> DNA GE/ml</td>
</tr>
<tr>
<td>X x 10⁹ GE/ml</td>
<td>Calculated concentration value (copies/ml) is in the lower limit of measurement range of the PCR kit. The result is <em>Streptococcus pyogenes</em> DNA is detected in concentration X x 10⁹ copies/ml</td>
</tr>
</tbody>
</table>
Streptococcus pyogenes DNA was detected in concentration greater than the upper limit of measurement range of the PCR kit. The result is greater than $1 \times 10^7$ Streptococcus pyogenes DNA GE/ml. If the accurate quantification is required, the extracted sample is to be diluted by TE-buffer reagent (for example, 100-fold dilution) and the PCR-analysis is to be repeated from the amplification stage. The result obtained after repeated analysis should be multiplied by the coefficient of the sample dilution.

Boundary Ct values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

The results of the analysis is considered reliable only if the results obtained for controls of amplification and extraction stages are correct (according to Table 5 and the *Important Product Information Bulletin* enclosed to the PCR kit).

<table>
<thead>
<tr>
<th>Control</th>
<th>Stage for control</th>
<th>FAM</th>
<th>JOE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-</td>
<td>DNA extraction</td>
<td>$Ct$ value $&lt;$ boundary value</td>
<td>$Ct$ value is absent</td>
</tr>
<tr>
<td>PCE</td>
<td>DNA extraction</td>
<td>$Ct$ value $&lt;$ boundary value</td>
<td>$Ct$ value $&lt;$ boundary value, concentration value is within the range</td>
</tr>
<tr>
<td>NCA</td>
<td>PCR</td>
<td>$Ct$ value is absent</td>
<td>$Ct$ value is absent</td>
</tr>
<tr>
<td>C1</td>
<td>PCR</td>
<td>$Ct$ value and calculated concentration are defined</td>
<td>$Ct$ value and calculated concentration are defined</td>
</tr>
<tr>
<td>C2</td>
<td>PCR</td>
<td>$Ct$ value and calculated concentration are defined</td>
<td>$Ct$ value and calculated concentration are defined</td>
</tr>
</tbody>
</table>

Boundary Ct values and the range of Positive Control *Streptococcus pyogenes* concentration are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

**10. TROUBLESHOOTING**

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

1. The $Ct$ value determined for the Positive Control of Extraction (PCE) in the channels for the FAM and/or JOE fluorophores is greater than the boundary $Ct$ value or absent. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples.

2. The calculated concentration of the Positive Control *Streptococcus pyogenes* does not fit in the range specified in the *Important Product Information Bulletin*. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples.

3. The $Ct$ value is determined for the Negative Control of Extraction (C–) in the channel for the JOE fluorophore. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis.
Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which specific DNA was detected.

4. The Ct value is determined for the Negative Control of amplification (NCA) in the channels for the FAM and/or JOE fluorophores. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The amplification and detection should be repeated for all samples in which specific DNA was detected.

5. The Ct values and calculated concentration are absent for the DNA-calibrators C1 and C2 in either of the specified channels for fluorophores. The amplification and detection should be repeated for all the samples.

6. The correlation coefficient R² is less than 0.98 when plotting the calibration curve. Check the correctness of set concentrations of calibrators in accordance with the Important Product Information Bulletin. If the improper result has been obtained again the amplification and detection for all the samples should be repeated.

7. The Ct value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level or parameters of base line calculation. If the result has been obtained with the correct level of threshold line (base line), the amplification and detection should be repeated for this sample.

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

11. TRANSPORTATION
AmpliSens® Streptococcus pyogenes-screen/monitor-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® Streptococcus pyogenes-screen/monitor-FRT PCR kit are to be stored at 2–8 °C when not in use (except for PCR-buffer-H and PCR-mix-FL Streptococcus pyogenes). All components of the AmpliSens® Streptococcus pyogenes-screen/monitor-FRT PCR kit are stable until labeled expiration date. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

⚠️ PCR-buffer-H and PCR-mix-FL Streptococcus pyogenes are to be stored at the temperature from minus 24 to minus 16 °C
PCR-mix-FL *Streptococcus pyogenes* is to be kept away from light

PCR-mix *Streptococcus pyogenes*-Lyo is to be kept in packages with a desiccant away from light

### 13. SPECIFICATIONS

#### 13.1. Measurement range and limit of detection

<table>
<thead>
<tr>
<th>Test material</th>
<th>Transport medium</th>
<th>Nucleic acid extraction kit</th>
<th>PCR kit</th>
<th>Limit of detection, GE/ml</th>
<th>Measurement range, GE/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oropharyngeal swabs</td>
<td>Transport Medium for Storage and Transportation of Respiratory Swabs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue (biopsy, surgical, autopsy) material</td>
<td>Transport Medium with Mucolytic Agent</td>
<td>RIBO-prep</td>
<td>PCR kit variant FRT-100 FN, PCR kit variant FRT-L</td>
<td>4x10²</td>
<td>1x10³ – 1x10⁷</td>
</tr>
<tr>
<td>Cerebrospinal fluid (CSF)</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synovial fluid</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discharge of erosive and ulcerative lesions of the skin</td>
<td>Transport Medium with Mucolytic Agent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The claimed features are achieved while respecting the rules specified in the section *Sampling and Handling*.

#### 13.2. Analytical specificity

The analytical specificity of *AmpliSens® Streptococcus pyogenes*-screen/monitor-FRT PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The PCR kit detects the DNA fragments of claimed microorganisms. The analytical specificity was proved on the following strains of microorganisms *Acinetobacter baumanii ATCC® 19606™, Enterococcus faecalis ATCC® 29212™, Escherichia coli ATCC® 25922™, Haemophilus influenzae ATCC® 33930™, Haemophilus haemolyticus, Haemophilus parainfluenzae, Klebsiella pneumoniae ATCC® 27736™, Klebsiella oxytoca, Listeria grayi (murrayi) ATCC® 25401™, Listeria innocua ATCC® 33090™, Listeria monocytogenes ATCC® 7644™, Pseudomonas aeruginosa ATCC® 15442™, Staphylococcus aureus ATCC® 6538P™, Staphylococcus aureus (MRSA) ATCC® 43300™, Staphylococcus epidermidis ATCC® 12228™, Staphylococcus haemolyticus ATCC® 29970™,
Staphylococcus saprophyticus ATCC® 49907™, Streptococcus agalactiae ATCC® 12386™, Streptococcus milleri, Streptococcus sanguis, Streptococcus mitis, Moraxella catarrhalis ATCC® 25240™, Proteus mirabilis, Neisseria sicca, Neisseria flava, Neisseria subflava, Neisseria cinerea, Neisseria mucosa, Neisseria elongata, Neisseria gonorrhoeae, Neisseria meningitidis (gr. B), Neisseria meningitidis (gr. W), Neisseria meningitidis (gr. Y), Candida albicans, Candida glabrata, Candida parapsilosis, Pneumocystis jiroveci (carinii), Toxoplasma gondii, HSV1 (Herpes simplex virus type 1), HSV2 (Herpes simplex virus type 2), HCMV (human cytomegalovirus), EBV (Epstein-Barr virus), HHV6 (human herpesvirus type 6), HHV7 (human herpesvirus type 7), VZV (varicella zoster virus), JCV (JC virus), BKV (BK virus), Parvovirus B19, Enterovirus and also human genomic DNA.

The nonspecific responses were not observed while testing the DNA samples of the above mentioned microorganisms, as well as human DNA.

The PCR kit detects the DNA fragments of Streptococcus pyogenes: Streptococcus pyogenes ATCC® 19615™

The clinical specificity of AmpliSens® Streptococcus pyogenes-screen/monitor-FRT PCR kit was confirmed in laboratory clinical trials.

13.3. Reproducibility, repeatability and trueness

Repeatability and reproducibility were determined by testing of quality control sample (QCS Positive Control Streptococcus pyogenes DNA) with concentrations 1x10⁶; 1x10⁵ and 1x10⁴ GE/ml which lay within the measurement range.

<table>
<thead>
<tr>
<th>Reproducibility</th>
<th>PCR kit</th>
<th>Initial concentration value, GE/ml</th>
<th>Number of repeats</th>
<th>Average concentration value, lg</th>
<th>Standard deviation (SD)</th>
<th>Coefficient of variation (CV), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR kit variant</td>
<td>FRT-100</td>
<td>1x10⁶</td>
<td>80</td>
<td>6,07</td>
<td>0,05</td>
<td>0,83</td>
</tr>
<tr>
<td>FN</td>
<td></td>
<td>1x10⁵</td>
<td>80</td>
<td>5,12</td>
<td>0,05</td>
<td>1,01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1x10⁴</td>
<td>80</td>
<td>4,12</td>
<td>0,06</td>
<td>1,45</td>
</tr>
<tr>
<td>PCR kit variant</td>
<td>FRT-L</td>
<td>1x10⁶</td>
<td>80</td>
<td>6,11</td>
<td>0,08</td>
<td>1,29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1x10⁵</td>
<td>80</td>
<td>5,16</td>
<td>0,09</td>
<td>1,71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1x10⁴</td>
<td>80</td>
<td>4,13</td>
<td>0,09</td>
<td>2,21</td>
</tr>
</tbody>
</table>
Table 7

<table>
<thead>
<tr>
<th>PCR kit variant</th>
<th>Initial concentration value, GE/ml</th>
<th>Number of repeats</th>
<th>Average concentration value, lg</th>
<th>Standard deviation (SD)</th>
<th>Coefficient of variation (CV), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR kit variant FRT-100 FN</td>
<td>$1 \times 10^6$</td>
<td>40</td>
<td>6,05</td>
<td>0,03</td>
<td>0,48</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^5$</td>
<td>40</td>
<td>5,03</td>
<td>0,04</td>
<td>0,84</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^4$</td>
<td>40</td>
<td>4,05</td>
<td>0,05</td>
<td>1,14</td>
</tr>
<tr>
<td>PCR kit variant FRT-L</td>
<td>$1 \times 10^6$</td>
<td>40</td>
<td>6,07</td>
<td>0,03</td>
<td>0,54</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^5$</td>
<td>40</td>
<td>5,07</td>
<td>0,05</td>
<td>0,89</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^4$</td>
<td>40</td>
<td>4,09</td>
<td>0,06</td>
<td>1,39</td>
</tr>
</tbody>
</table>

Trueness was determined by testing of quality control sample (QCS Positive Control *Streptococcus pyogenes* DNA) with known concentration.

Table 8

<table>
<thead>
<tr>
<th>PCR kit variant</th>
<th>Number of repeats</th>
<th>Average concentration value, lg</th>
<th>Specified value, lg</th>
<th>Bias (B), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR kit variant FRT-100 FN</td>
<td>100</td>
<td>6,78</td>
<td>6,78</td>
<td>0,00</td>
</tr>
<tr>
<td>PCR kit variant FRT-L</td>
<td>100</td>
<td>6,72</td>
<td>6,78</td>
<td>0,91</td>
</tr>
</tbody>
</table>

13.4. Diagnostic characteristics

The samples of biological material (oropharyngeal swabs, whole blood, autopsy material, synovial fluid, discharge of erosive and ulcerative lesions of the skin, cerebrospinal fluid (CSF), urine) taken from the persons suspected of streptococcal infection were used to confirm the diagnostic specificity of AmpliSens® *Streptococcus pyogenes* screen/monitor-FRT PCR kit. The absence of streptococcal infection was confirmed by biological methods.

To confirm diagnostic sensitivity of AmpliSens® *Streptococcus pyogenes* screen/monitor-FRT PCR kit the above-mentioned samples were artificially contaminated by collection strain *Streptococcus pyogenes* ATCC® 19615™ of American type culture collection (ATCC).
Table 9

The results of testing AmpliSens® *Streptococcus pyogenes*-screen/monitor-FRT PCR kit

<table>
<thead>
<tr>
<th>Sample type</th>
<th>The results of application of AmpliSens® <em>Streptococcus pyogenes</em>-screen/monitor-FRT PCR kit</th>
<th>Positive samples contaminated with <em>Streptococcus pyogenes</em> (ATCC® 19615™)</th>
<th>Negative samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oropharyngeal swabs</td>
<td>200 samples were tested</td>
<td>Positive 100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative 0</td>
<td>100</td>
</tr>
<tr>
<td>Whole blood</td>
<td>200 samples were tested</td>
<td>Positive 100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative 0</td>
<td>100</td>
</tr>
<tr>
<td>Autopsy material</td>
<td>200 samples were tested</td>
<td>Positive 100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative 0</td>
<td>100</td>
</tr>
<tr>
<td>Synovial fluid</td>
<td>200 samples were tested</td>
<td>Positive 100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative 0</td>
<td>100</td>
</tr>
<tr>
<td>Discharge of erosive and ulcerative lesions of the skin</td>
<td>200 samples were tested</td>
<td>Positive 100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative 0</td>
<td>100</td>
</tr>
<tr>
<td>Cerebrospinal fluid (CSF)</td>
<td>200 samples were tested</td>
<td>Positive 100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative 0</td>
<td>100</td>
</tr>
<tr>
<td>Urine</td>
<td>200 samples were tested</td>
<td>Positive 100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative 0</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 10

Diagnostic characteristics of AmpliSens® *Streptococcus pyogenes*-screen/monitor-FRT PCR kit

<table>
<thead>
<tr>
<th>Тип образцов</th>
<th>Diagnostic sensitivity, no less than %</th>
<th>Diagnostic specificity, no less than %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oropharyngeal swabs</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Whole blood</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Autopsy material</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Synovial fluid</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Discharge of erosive and ulcerative lesions of the skin</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cerebrospinal fluid (CSF)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Urine</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

14. REFERENCES

1. Guidelines to AmpliSens® *Streptococcus pyogenes*-screen/monitor-FRT PCR kit using the PCR instruments with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”

15. QUALITY CONTROL

In accordance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Total Quality Management System, each lot of
AmpliSens® *Streptococcus pyogenes*-screen/monitor-FRT PCR kit is tested against predetermined specifications to ensure consistent product quality.
### 16. KEY TO SYMBOLS USED

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>IVD</td>
<td><em>In vitro</em> diagnostic medical device</td>
</tr>
<tr>
<td>VER</td>
<td>Version</td>
</tr>
<tr>
<td></td>
<td>Temperature limitation</td>
</tr>
<tr>
<td></td>
<td>Manufacturer</td>
</tr>
<tr>
<td></td>
<td>Date of manufacture</td>
</tr>
<tr>
<td></td>
<td>Authorised representative in the European Community</td>
</tr>
<tr>
<td></td>
<td>Positive control of extraction</td>
</tr>
<tr>
<td>PCE</td>
<td>DNA-calibrators</td>
</tr>
<tr>
<td>IC</td>
<td>Internal control</td>
</tr>
</tbody>
</table>

**Caution**
- Keep away from sunlight
- Consult instructions for use
- Negative control of extraction
- Positive control of amplification