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For Professional Use Only

AmpliSens® *Streptococcus pyogenes*-screen-titre-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *Streptococcus pyogenes*-screen-titre-FRT PCR kit is an *in vitro* nucleic acid amplification test for detection and quantitative analysis of the *Streptococcus pyogenes* DNA in the clinical material (throat swabs, sputum, blood, biopsies, synovial fluid, wound discharge, cerebrospinal fluid (CSF), and urine) using PCR amplification with real-time fluorescence-hybridization detection of amplified products.

The results of PCR analysis are taken into account in complex diagnostics of disease[1].

2. PRINCIPLE OF PCR DETECTION

Streptococcus pyogenes DNA detection by the polymerase chain reaction (PCR) with fluorescence-hybridization detection consists of the two stages: the DNA extraction from the clinical material samples and the DNA amplification of the DNA region of that microorganism using fluorescence hybridization detection, which is conducted simultaneously with the PCR. DNA extraction from the clinical material sample is carried out in the presence of the **Internal Control STI-87 (IC)** that allows controlling the execution of the analysis procedure for each sample. Upon DNA extraction from the clinical material, which contains cells, the amplification of the DNA region from the human genome (the endogenous internal control) is also carried out. The endogenous internal control (**IC Glob**) permits not only to control the PCR analysis stages (DNA extraction and execution of PCR), but also to evaluate the adequacy of material collection and its storage. Then, the obtained DNA samples are used to amplify the *Streptococcus pyogenes* DNA region using specific for that region DNA primers and the Taq-polymerase enzyme. The reaction mix contains fluorescently labeled oligonucleotide markers, which hybridize to complementary regions of the target DNA being amplified; as a result the accumulation of the fluorescence intensity is observed. The fluorescence signal detection is conducted directly during the PCR execution with the aid of the fluorescence signal detection system in “real-time” mode.

AmpliSens® *Streptococcus pyogenes*-screen-titre-FRT PCR kit uses “hot-start”, which greatly reduces the frequency of non-specifically primed reactions. “Hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase using chemically

[1] In compliance with EU Directive 98/79/EC

modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENTS

AmpliSens® *Streptococcus pyogenes*-screen-titre-FRT PCR kit is produced in 1 form:

AmpliSens® *Streptococcus pyogenes*-screen-titre-FRT PCR kit variant FRT-100 F,

REF R-B82-100-FT(RG,iQ)-CE

AmpliSens® *Streptococcus pyogenes*-screen-titre-FRT PCR kit variant FRT-100 F includes:

Reagent		*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *Streptococcus pyogenes*-screen-titre		colorless clear liquid	1.2	1 tube
PCR-mix-2-FRT		colorless clear liquid	0.6	1 tube
Polymerase (TaqF)		colorless clear liquid	0.06	1 tube
TE-buffer		colorless clear liquid	0.5	1 tube
DNA calibrators	**K1 *SPG***	colorless clear liquid	0.2	1 tube
	K2 *SPG*	colorless clear liquid	0.2	1 tube
Negative Control (C-)*		colorless clear liquid	1.2	1 tube
Positive Control DNA *Streptococcus pyogenes* and human DNA**		colorless clear liquid	0.2	1 tube
Internal Control STI-87 (IC)***		colorless clear liquid	1.0	1 tube

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

** must be used in the extraction procedure as Positive Control of Extraction (see RIBO-prep **REF** K2-9-Et-100-CE protocol).

*** add 10 µl of Internal Control during the DNA extraction procedure directly to the sample/lysis mixture (see RIBO-prep, **REF** K2-9-Et-50-CE protocol).

AmpliSens® *Streptococcus pyogenes*-screen-titre-FRT PCR kit is intended for 110 reactions (including controls and calibrators).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit or DNA extraction automatic station.
- Disposable powder-free gloves and a laboratory coat.

- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (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 iQ5 (Bio-Rad, USA))
- Disposable polypropylene PCR tubes:
    a) 0.2-ml PCR tubes with optically 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 with the temperature range from 2 to 8 °°C.
- Deep-freezer with the temperature 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 barriers 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 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 specimens or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.

- Avoid specimens and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Use the disposable pipette filter tips for each operation.
- The laboratory surfaces and facilities on which the PCR analysis is carried out are subject to ultraviolet radiation for 30 min prior to and after the analysis.
- Material Safety Data Sheets (MSDS) 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.

When disposing of waste products after amplification (test tubes with PCR products) it is necessary to avoid opening the tubes or spilling the tube contents, as that may lead to PCR products contamination of the laboratory area, instruments and reagents.

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® *Streptococcus pyogenes*-screen-titre-FRT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material: throat swabs, sputum, blood, biopsies, synovial fluid, wound discharge, cerebrospinal fluid (CSF), and urine.

Pretreatment

6.1 *Blood plasma* – tubes filled with whole blood are placed into the centrifuge at **800 g** for **10 min** at room temperature. Collect at least 1 ml of blood plasma from each sample with individual pipette tips with aerosol filters, and transfer the plasma into sterile 1.5-2.0 ml tubes. Centrifuge the tubes containing 1 ml of blood plasma for further 10-20 min at 7,000 g (11,000 rpm). For extraction use the sediment and 100 µl of over-sedimentary liquid.

7. WORKING CONDITIONS

AmpliSens® *Streptococcus agalactiae*-screen-titre-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-50-CE;

– NucliSENS easyMAG automated system (for details see *Guidelines* [2]).

DNA extraction from each clinical material sample is conducted in the presence of the **Internal Control STI-87 (IC)** (add **10 µl** of **Internal Control STI-87 (IC)** to each sample). Add **100 µl** of **Negative Control (C-)** to the tube labeled **Negative Control (C-)**. Add **90 µl** of **Negative Control (C-)** and **10 µl** of **Positive Control DNA *Streptococcus pyogenes* and human DNA** to the tube labeled Positive Control of Extraction (PCE).

Extract the DNA according to the manufacturer's protocol.

In case of extracting DNA using NucliSENS easyMAG (bioMérieux, France) automatic station, please, consult the **AmpliSens® *Streptococcus pyogenes*-screen-titre-FRT** PCR kit *Guidelines* [2] for information about the procedure.

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

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

Tube selection depends on the thermocycler with "real-time" detection system being used.

1. Prior to the experiment prepare a mix of **PCR-mix-2-FRT** and **Polymerase (TaqF)**. Transfer the **Polymerase (TaqF)** tube contents (**60 µl**) to the **PCR-mix-2-FRT** tube (**600 µl**) and gently vortex avoiding the formation of foam. Mark the tube with the reaction mix preparation date.

Prepared mix is intended for 120 samples. Store the samples at a temperature range of 2 to 8 °°C for 3 months, to be used upon the need. If the prepared mix can not be consumed in the course of 3 months, then prepare the mix for smaller number of reactions, for example, add **150 µl** of **PCR-mix-2-FRT** and **15 µl** of **Polymerase (TaqF)** (prepared mix is intended for 30 reactions)

2. Prepare the reaction mix. Take into account that in order to test one DNA sample in <u>qualitative</u> format, <u>two</u> controls of PCR amplification must be set up – Positive Control

(**K2 *SPG*** DNA calibrator) and Negative Control (**TE-buffer**); and in quantitative format, five controls of PCR amplification must be set up: two DNA calibrators (***K1 SPG*** and ***K2 SPG***) with two repeats and Negative Control of PCR (**TE-buffer**). It is necessary to take reagents in reserve and calculate the volumes including 1 extra reaction.

3. In a separate tube mix **PCR-mix-1-FL *Streptococcus pyogenes*-screen-titre** with the previously prepared mix of **PCR-mix-2-FRT** and **Polymerase (TaqF)**. Calculation is based on the fact that each PCR loading includes:

   - **10 µl** of **PCR-mix-1-FL *Streptococcus pyogenes*-screen-titre**
   - **5 µl** of **PCR-mix-2-FRT** and **Polymerase (TaqF)** mix

To make calculations for the required number of reactions, including testing of analyzed and control samples, consult Table 1 of the *Instruction manual*.

In simultaneous analysis of 120 samples, a simplified mix preparation scheme can be used: transfer all contents from one **PCR-mix-2-FRT** tube and all contents from one **Polymerase (TaqF)** tube to the **PCR-mix-1-FL *Streptococcus pyogenes*-screen-titre** tube.

Table 1

Scheme of reaction mixture preparation for variant FRT-100 F

Reagent volume for specified number of reactions, total reaction volume – 25 µl, including 10 µl of the DNA sample.			
Reagent volume per one reaction, µl		**10,0**	**5,0**
Number of clinical samples		**PCR-mix-1-FRT *Streptococcus pyogenes*-screen-titre[2]**	**Mix of PCR-mix-2-FRT and polymerase (TaqF)[2]**
for qualitative analysis	**for quantitative analysis**		
1	**4**	**70**	**35**
2	**5**	**80**	**40**
3	**6**	**90**	**45**
4	**7**	**100**	**50**
5	**8**	**110**	**55**
6	**9**	**120**	**60**
7	**10**	**130**	**65**
8	**11**	**140**	**70**
9	**12**	**150**	**75**
10	**13**	**160**	**80**
11	**14**	**170**	**85**
12	**15**	**180**	**90**
13	**16**	**190**	**95**
14	**17**	**200**	**100**
15	**18**	**210**	**105**
16	**19**	**220**	**110**
17	**20**	**230**	**115**
18	**21**	**240**	**120**
19	**22**	**250**	**125**
20	**23**	**260**	**130**
21	**24**	**270**	**135**
22	**25**	**280**	**140**
23	**26**	**290**	**145**
24	**27**	**300**	**150**
25	**28**	**310**	**155**
30	**33**	**360**	**180**

4. Select the required number of tubes for amplification of the analyzed and control DNA samples.
5. Add **15 µl** of the reaction mix to each tube.
6. Add **10 µl** of the analyzed DNA, obtained by the extraction from clinical and control

[2] Values are given including the reserve (volume calculation for 1 extra reaction) and including five controls (2 DNA calibrators K1 *SPG* and K2 SPG (both repeated) for quantitative analysis of *Streptococcus pyogenes* DNA and two controls (positive and negative controls) for qualitative analysis of *Streptococcus pyogenes* DNA.

samples, to the reaction mix tubes.

7. Carry out the control amplification reactions:

For qualitative analysis:

NCA - Add **10 µl** of **TE-buffer** to the tube labeled NCA.

C+ - Add **10 µl** of **K2 *SPG* DNA calibrator** to the tube labeled C+

For quantitative analysis:

NCA - Add **10 µl** of **TE-buffer** to the tube labeled NCA.

K1 *SAG* - Add **10 µl** of **K1 *SPG* DNA calibrator** to two tubes

K2 *SAG* - Add **10 µl** of **K2 *SPG* DNA calibrator** to two tubes

8.2.2. Amplification

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

Table 2

"AmpliSens-1" amplification program

	Rotor-type instruments[3]			***Plate-type instruments***[4]		
Step	***Temperature, °C***	***Time***	***Cycles***	***Temperature, °C***	***Time***	***Cycles***
1	**95**	**15 min**	1	**95**	**15 min**	1
2	**95**	5 s	5	**95**	5 s	5
	60	20 s		**60**	20 s	
	72	15 s		**72**	15 s	
3	**95**	5 s	40	**95**	5 s	40
	60	20 s Fluorescence detection		**60**	30 s Fluorescence detection	
	72	15 s		**72**	15 s	

The fluorescence signal is detected in the channels for the FAM, JOE and ROX fluorophores.

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. Analyse and interpret 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 three channels:

[3] For example, Rotor-Gene 3000/Rotor-Gene 6000 (Corbett Research, Australia)
[4] For example, iCycler iQ, iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA).

– Channel for the **FAM** fluorophore (or analogous, depending on the instrument in use) registers the signal testifying the accumulation of the β-globin gene fragment DNA (**IC Glob)** amplification product
– Channel for the **JOE** fluorophore (or analogous, depending on the instrument in use) registers the signal testifying the accumulation of ***Streptococcus pyogenes*** DNA amplification product.
– Channel for **ROX** fluorophore (or analogous, depending on the instrument in use) registers a signal testifying the accumulation of amplification product of **Internal Control STI-87 (IC)** DNA.

Upon DNA extraction from blood plasma, synovial fluid, cerebrospinal fluid (CSF) and urine the results for two channels are considered: the channel for the **JOE** fluorophore - ***Streptococcus pyogenes*** DNA, the channel for the **ROX** fluorophore registers the signal testifying the accumulation of amplification product of the **Internal Control STI-87 (IC)** DNA.

Upon DNA extraction from blood, throat swabs, sputum, biopsies and wound discharge the results for two channels are considered: the channel for the **FAM** fluorophore registers a signal testifying the accumulation of amplification product for the β-globin gene DNA (**IC Glob**), and the channel for the **JOE** fluorophore - ***Streptococcus pyogenes*** DNA.

Results are interpreted by the presence (or absence) of an intercept between the fluorescence curve and 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 table.

Principle of interpretation of results for the DNA extraction from blood plasma, synovial fluid, cerebrospinal fluid (CSF) and urine is the following:

– *Streptococcus pyogenes* DNA is **detected** if the *Ct* value determined in the results table in the channel for the JOE fluorophore is less than the boundary *Ct* value. Moreover, the fluorescence curve of the sample should intercept the threshold line in the area of characteristic exponential increase of fluorescence intensity.
– *Streptococcus pyogenes* DNA is **not detected** in a sample if the *Ct* value is not determined (absent) in the channel for **JOE** fluorophore (fluorescence curve does not intercept the threshold line), and in the results table for the channel for the **ROX** fluorophore the *Ct* value is determined as less than the boundary *Ct* value.
– The result is **invalid** if the *Ct* value is not determined (absent) for the given sample

in the channel for **JOE** fluorophore, and in the channel for **ROX** fluorophore the *Ct* value is also not determined (absent) or is greater than the specified boundary *Ct* value. In such cases, the PCR analysis should be repeated for the corresponding clinical sample.

– For the clinical samples in which the *Ct* values in the channel for the **JOE** fluorophore are determined as greater than the boundary *Ct* value, the results are considered **equivocal**. In such cases, the PCR analysis should be repeated for the given sample with two repeats. In case of obtaining a reproducible positive *Ct* value, the result is interpreted as positive. If the results obtained for two repeats are not reproducible, the result is interpreted as **equivocal**.

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

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of extraction and of amplification are correct (see Table 3).

For quantitative analysis the results for Positive Control of Extraction (PCE) must be within the concentrations range outlined in the *Important Product information Bulletin* enclosed to the PCR kit.

Table 3

Results for controls of different stages of PCR analysis for DNA extraction from blood plasma, synovial fluid, cerebrospinal fluid (CSF) and urine is the following:

Control	Stage for control	Amplification results in the channel for fluorophore			
		JOE		ROX	
		Qualitative format	Quantitative format	Qualitative format	Quantitative format
C–	DNA extraction	Absent	Absent	< boundary value	< boundary value
PCE	DNA extraction	< boundary value	Obtained value is within the range defined in the *Important Product Information Bulletin*	< boundary value	< boundary value
NCA	PCR	Absent	Absent	Absent	Absent
C+	PCR	**<**boundary value	**–**	**<**boundary value	**–**
K1 *SPG* K2 *SPG*	PCR	–	*Ct* value and calculated concentrations are defined	–	*Ct* value and calculated concentrations are defined

Principle of interpretation of results for the DNA extraction from blood, throat swabs, sputum, biopsies and wound discharge is the following:

- *Streptococcus pyogenes* DNA is **detected** if the *Ct* value determined in the results table in the channel for the **JOE** fluorophore is less than the boundary *Ct* value. Moreover, the fluorescence curve of the given sample must intercept the threshold line in the area of characteristic exponential increase of fluorescence intensity.
- *Streptococcus pyogenes* DNA is **not detected** in a sample if the *Ct* value is not determined (absent) in the channel for **JOE** fluorophore (fluorescence curve does not intercept the threshold line), and in the results table for the channel for the **FAM** fluorophore for qualitative format the *Ct* value is determined that does not exceed the boundary *Ct* value, or, similarly, the number of human genomes (**IC Glob**) for the reaction does not exceed **500** (in quantitative analysis).
- The result is **invalid** if the *Ct* value is not determined (absent) for the given sample in the channel for **JOE** fluorophore, and in the channel for the **FAM** fluorophore for qualitative format the Ct value is also not determined (absent) or is greater than the boundary *Ct* value (qualitative format), and for quantitative format – the number of human genome equivalents (**IC Glob**) for the reaction is less than **500**. In such cases, the PCR analysis should be repeated for the corresponding clinical sample.

– For the clinical samples in which the *Ct* values in the channel for the **JOE** fluorophore are determined as greater than the boundary *Ct* value the results are considered **equivocal**. In such cases, the PCR analysis should be repeated for the given sample with two repeats. In case of obtaining the reproducible positive *Ct* value, the result is interpreted as positive. If the results obtained are not reproducible, the result is interpreted as **equivocal**.

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

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of extraction and of amplification are correct (seeTable 4).

For quantitative analysis the results for PCE must be within the concentrations range outlined in the *Important Product Information Bulletin* enclosed with the PCR kit.

Table 4

Results for controls of different stages of PCR analysis for DNA extraction from blood, throat swabs, sputum, biopsies and wound discharge

Control	Stage for control	Amplification results in the channel for fluorophore			
		JOE		ROX	
		Qualitative format	Quantitative format	Qualitative format	Quantitative format
C–	DNA extraction	Absent	Absent	Absent	Absent
PCE	DNA extraction	< boundary value	Obtained value is within the range defined in the *Important Product Information Bulletin*	< boundary value	< boundary value
NCA	PCR	Absent	Absent	Absent	Absent
C+	PCR	**<**boundary value	**–**	**<**boundary value	**–**
K1 *SAG* K2 *SAG*	PCR	–	*Ct* value and calculated concentration are defined	–	*Ct* value and calculated concentration are defined

For quantitative analysis the calculation of *Streptococcus pyogenes* DNA concentration in 1 ml of the sample should use the following formula:

Calculated *Streptococcus pyogenes* DNA concentration x coefficient A x 100 = copies/ml.

$$\textit{Coefficient A} = \frac{\textit{100}}{\textit{extract volume, µl}}$$

10. TROUBLESHOOTING

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

1. If the positive signal is absent in the DNA calibrators, it may suggest that the amplification program is chosen wrongly and that other mistakes were made at the loading of PCR stage. In such case, repeat the PCR analysis for all samples.
2. If for the Negative Control of Extraction (C-) and/or for the Negative Control of Amplification (NCA) in the channel for the **JOE** fluorophore, the Ct value is detected, then repeat the analysis of the positive probes starting with the extraction stage, and undertake the necessary actions to identify the source of contamination.
3. If for the tested sample a positive signal is detected, but the fluorescence curve is missing the region of characteristic exponential increase (the curve represents a more of a straight line), that may suggest that the threshold level or the basal line calculation parameters were incorrectly set up. Such result should not be interpreted as positive. If the result is obtained with the correctly set threshold level, it is necessary to repeat the PCR analysis for that 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-titre-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-titre-FRT** PCR kit are to be stored at 2–8°°C when not in use. All components of the **AmpliSens® *Streptococcus pyogenes*-screen-titre-FRT** PCR kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FL *Streptococcus pyogenes*-screen-titre
PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at the temperature range from minus 24 to minus 16°°C

PCR-mix-1-FL *Streptococcus pyogenes*-screen-titre is to be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

Clinical material	DNA extraction kit	Amplification and detection kit	Analytical sensitivity, copies/ml	Linear measuring range, copies/ml
Throat swabs, sputum, biopsies, synovial fluid, wound discharge, urine, cerebrospinal fluid (CSF)	RIBO-prep	PCR kit variant FRT-100 F	$3x10^2$	1,000-10,000,000

13.2. Specificity

The analytical specificity of **AmpliSens® *Streptococcus pyogenes*-screen-titre-FRT** PCR kit is ensured by the 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 analytical specificity is studied with the use of DNA/RNA strains and isolates of the following pathogens: *Candida albicans*, *Chlamydophila pneumonia*, *Cryptococcus neoformans*, *Cytomegalovirus hominis*, *Epstein–Barr virus* (*EBV*), *Escherichia coli*, *Haemophilus haemolyticus*, *H.influenzae*, *H.parainfluenzae*, *Hepatitis A virus* (*HAV*), *Hepatitis B virus* (*HBV*), *Hepatitis C virus* (*HCV*), *Hepatitis D virus* (*HDV*), *Herpes simplex virus I* (*HSV I*), *Herpes simplex virus II* (*HSV II*), *Human Herpes virus VI* (*HHV 6*), *Human Herpes virus VII* (*HHV 7*), *Human Herpes virus VIII* (*HHV 8*), *Human adenovirus B*, *C*, *E*, *F*; *Human immunodeficiency virus* (*HIV*), *Human papillomavirus 6, 11, 16, 18, 33, 35 (HPV 6, 11, 16, 18, 33, 35), Klebsiella oxytoca*, *K.pneumonia*, *Listeria monocytogenes*, *Measles virus*, *Moraxella catarrhalis*, *Mumps virus*, *Mycobacterium tuberculosis*, *Mycoplasma pneumonia*, *Neisseria cinereae*, *N.elongata*, *N.flavescens*, *N.gonorrhoeae*, *N.meningitidis*, *N.mucosa*, *N.sicca*, *N.subflava*, *Proteus mirabilis*, *P.vulgaris*, *Pseudomonas aeruginosa*, *Rubella virus*, *Salmonella typhimurium*, *Shigella flexneri*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *S.milleri*, *S.mitis*, *S.mutans*, *S.oralis, S.pneumoniae*, *S.salivarius*, *S.sanguis*, *S.suis*, *S.viridans*, *Toxoplasma gondii*, *Varicella–Zoster virus*, and

also human DNA. Nonspecific reactions were not detected whilst testing the DNA/RNA strains and isolates with the PCR kit.

The clinical specificity of **AmpliSens®** ***Streptococcus pyogenes*****-screen-titre-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, 2010.
2. Guidelines to the **AmpliSens®** ***Streptococcus pyogenes*-screen-titre-FRT** PCR kit for detection and quantitative analysis of the *Streptococcus pyogenes* DNA in the clinical material (throat swabs, sputum, blood, biopsies, synovial fluid, wound discharge, cerebrospinal fluid (CSF), and urine) using PCR amplification with real-time fluorescence-hybridization detection of amplified products.

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 the **AmpliSens®** ***Streptococcus pyogenes*-screen-titre-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+**	Positive control of amplification
IC	Internal control	**PCE**	Positive Control of Extraction