AmpliSens® *Streptococcus agalactiae*-screen-titre-FRT PCR kit

CE IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Contains sufficient for <n> tests
LOT	Batch code		Use-by Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limit	NCA	Negative control of amplification
	Manufacturer	C–	Negative control of extraction
	Date of manufacture	C+	Positive control of amplification
EC REP	Authorized representative in the European Community	IC	Internal control
	Caution	PCE	Positive control of extraction

1. INTENDED USE

AmpliSens® *Streptococcus agalactiae*-screen-titre-FRT PCR kit is an *in vitro* nucleic acid amplification test for detection and quantitative analysis of *Streptococcus agalactiae* DNA in the clinical material (blood plasma, cerebrospinal fluid (CSF), oropharyngeal swabs, epithelial swabs of lateral vaginal walls, anorectal swabs) using the polymerase chain reaction (PCR) with real-time fluorescence hybridization detection.

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

2. PRINCIPLE OF PCR DETECTION

Streptococcus agalactiae DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Streptococcus agalactiae* primers. In the real-time PCR, the amplified product is detected with the use of the 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 DNA extraction of cell-free biological material (blood plasma, CSF) is conducted with the aid of the **Internal Control STI-87 (IC)**. It must be used in the extraction procedure in order to control the extraction process of each individual sample. During the DNA extraction of cell containing clinical material, the DNA of the human genome (Endogenous Internal Control) is being amplified. The Endogenous Internal Control (**IC Glob**) allows not only to control the test stages (DNA extraction and PCR amplification) but also to assess the adequacy of sampling and storage of clinical material.

AmpliSens® *Streptococcus agalactiae*-screen-titre-FRT PCR kit uses “hot-start”, which greatly reduces the frequency of non-specifically primed reactions. “Hot-start” is guaranteed by using 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	ROX
DNA-target	DNA fragment of β-globin gene (IC Glob)	*Streptococcus agalactiae* DNA	Internal Control STI-87L (IC) DNA
Target gene	β-globin gene	DNA section of the non-structural repeating sip gene (surface Sip protein)	Artificially synthesized sequence

3. CONTENT

AmpliSens® *Streptococcus agalactiae*-screen-titre-FRT PCR kit is produced in 1 form:
variant FRT-100 F, REF R-B77-100-FT(RG,iQ)-CE

Variant FRT-100 F includes:

Reagent		Description	Volume, ml	Quantity
PCR-mix-1-FL *Streptococcus agalactiae*		clear liquid from colorless to light lilac colour	1.2	1 tube
PCR-mix-2-FRT		colourless clear liquid	0.6	1 tube
Polymerase (TaqF)		colourless clear liquid	0.06	1 tube
TE-buffer		colourless clear liquid	0.5	1 tube
DNA calibrators	**K1 *SAG***	colourless clear liquid	0.2	1 tube
	K2 *SAG*	colourless clear liquid	0.2	1 tube
Positive Control DNA *Streptococcus agalactiae* and human DNA*		colourless clear liquid	0.1	1 tube
Negative Control (C–)**		colourless clear liquid	1.2	2 tubes
Internal Control STI-87 (IC)***		colourless clear liquid	0.6	2 tubes

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

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

*** add **10 µl** of **Internal Control STI-87 (IC)** during the DNA extraction procedure directly to the sample/lysis mixture (see **RIBO-prep**, REF K2-9-Et-50-CE protocol).

Variant FRT-100 F 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 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 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 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® *Streptococcus agalactiae*-screen-titre-FRT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (blood plasma, cerebrospinal fluid (CSF), oropharyngeal swabs, epithelial swabs of lateral vaginal walls, anorectal swabs)).

Pre-treatment

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 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 tube). Add **100 µl** of **Negative Control (C–)** to the tube labelled Negative **Control (C–)**. Add **90 µl** of **Negative Control (C–)** and **10 µl** of **Positive Control DNA *Streptococcus agalactiae* and human DNA** to the tube labelled Positive Control of Extraction (PCE).

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

NOTE: In case of extracting DNA using NucliSENS easyMAG (bioMérieux, France) automatic station, please, consult the **AmpliSens® *Streptococcus agalactiae*-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**.

NOTE: 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.

NOTE: 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 cannot 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 qualitative format, two controls of PCR amplification must be set up – Positive Control (**K2 *SAG*** DNA calibrator) and Negative Control (**TE-buffer**); and in quantitative format, five controls of PCR amplification must be set up: two DNA calibrators (***K1 SAG*** and ***K2 SAG***) 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 agalactiae*** 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 agalactiae***
   - **5 µl** of **PCR-mix-2-FRT** and **Polymerase (TaqF)** mix

To make calculations for the required number of reactions, including testing of analysed and control samples, see Table 2.

Table 2

Scheme of reaction mixture preparation for variant FRT-100 F

Reagent volume for specified number of reactions			
Reagent volume per one reaction, µl		10,0	5,0
Number of clinical samples		PCR-mix-1-FRT *Streptococcus agalactiae*[1]	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

NOTE: 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 agalactiae*** tube.

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.

NOTE: Pipette tips with aerosol filters are used for transfer of DNA and control samples.

6. Add **10 µl** of **DNA samples** extracted from test and control samples into the prepared tubes.

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

7. Carry out the control amplification reactions:

For qualitative analysis:

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

C+ – Add **10 µl** of **K2 *SAG* DNA calibrator** to the tube labelled C+

For quantitative analysis:

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

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

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

8.2.2. Amplification

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

Table 3

"AmpliSens 1" amplification program

	Rotor-type instruments[2]			Plate-type instruments[3]		
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	

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.

[1] Values are given including the reserve (volume calculation for 1 extra reaction) and including five controls (2 DNA calibrators K1 *SAG* and K2 SAG (both repeated) for quantitative analysis of *Streptococcus agalactiae* DNA and two controls (positive and negative controls) for qualitative analysis of *Streptococcus agalactiae* DNA.
[2] For example, Rotor-Gene 3000/Rotor-Gene 6000 (Corbett Research, Australia).
[3] For example, iCycler iQ, iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA).

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:

– channel for the **FAM** fluorophore registers the signal testifying the accumulation of the β-globin gene DNA (**IC Glob**) amplification product;
– channel for the **JOE** fluorophore registers the signal testifying the accumulation of ***Streptococcus agalactiae*** DNA amplification product;
– channel for **ROX** fluorophore registers a signal testifying the accumulation of amplification product of **Internal Control STI-87 (IC)** DNA.

Upon DNA extraction from oropharyngeal swabs, epithelial swabs of lateral vaginal walls, anorectal swabs, 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 agalactiae*** DNA.

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

– *Streptococcus agalactiae* 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 specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of the sample should intercept the threshold line in the area of characteristic exponential increase of fluorescence intensity.
– *Streptococcus agalactiae* 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 specified in the *Important Product Information Bulletin*.
– The result is **invalid** if the *Ct* value is not determined (absent) for the given sample in the channel for **JOE** fluorophore, whereas the *Ct* value in the channel for the **ROX** fluorophore is 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 specified in the *Important Product Information Bulletin* 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 are not reproducible, the result is interpreted as **equivocal**.

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 extraction and of amplification are correct (see Table 4). For quantitative analysis the results for Positive Control of Extraction (PCE) must be within the concentrations range outline in the *Important Product Information Bulletin* enclosed to the PCR kit.

Table 4

Results for controls of different stages of PCR analysis for DNA extraction from blood plasma and cerebrospinal fluid (CSF)

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 *SAG* K2 *SAG*	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 oropharyngeal swabs, epithelial swabs of lateral vaginal walls, anorectal swabs is the following:

– *Streptococcus agalactiae* 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 specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of the given sample must intercept the threshold line in the area of characteristic exponential increase of fluorescence intensity.
– *Streptococcus agalactiae* 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 as less than the boundary *Ct* value specified in the *Important Product Information Bulletin*, for quantitative format – the number of human genome equivalents (**IC Glob**) for the reaction is above **500**.
– 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 greater than the boundary *Ct* value specified in the *Important Product Information Bulletin*, and for quantitative format – the number of human genome equivalents (**IC Glob**) for the reaction is above **500**. In such cases, the PCR analysis should be repeated for the corresponding clinical sample starting from the DNA extraction stage.
– 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 specified in the *Important Product Information Bulletin* 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**.

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

Table 5

Results for controls of different stages of PCR analysis for DNA extraction from oropharyngeal swabs, epithelial swabs of lateral vaginal walls, anorectal swabs

Control	Stage for control	Amplification results in the channel for fluorophore			
		JOE		FAM	
		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	> boundary value	> boundary value
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 agalactiae* DNA concentration in one ml of the sample upon extraction from 100 ml should use the following formula:

Calculated *Streptococcus agalactiae* DNA concentration x 100 = copies/ml.

10. TROUBLESHOOTING

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

1. If the result for a sample is invalid, PCR analysis needs to be repeated for the corresponding clinical sample.
2. 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.
3. If in the Negative Control of Extraction (C-) in the channel for the JOE fluorophores or in the Negative Control of Amplification (NCA) in the channels for the JOE and/or ROX fluorophores, the positive signal is detected, then the reagents and the samples must have been contaminated. In this case, repeat the analysis of the positive probes starting with the extraction stage, and undertake the necessary actions to identify the source of contamination.
4. 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 agalactiae*-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 agalactiae*-screen-titre-FRT** PCR kit are to be stored at 2–8°°C when not in use. All components of the **AmpliSens® *Streptococcus agalactiae*-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.

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

NOTE: PCR-mix-1-FL *Streptococcus agalactiae* 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
Blood plasma, cerebrospinal fluid (CSF), oropharyngeal swabs, epithelial swabs of lateral vaginal walls, anorectal swabs	RIBO-prep	PCR kit variant FRT-100 F	$3x10^2$	1000-10,000,000

13.2. Specificity

The analytical specificity of **AmpliSens® *Streptococcus agalactiae*-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 reagents kit detects a fragment of *Streptococcus agalactiae* DNA. The specific activity of the reagents kit was confirmed by the analysis of *Streptococcus agalactiae* bacterial strains, and also by the analysis of clinical material with the followed confirmation of results using the methods of sequencing the amplification fragments.

The absence of kit components activity is shown with respect to the DNA of other bacterial pathogens (*Streptococcus pyogenes, Staphylococcus aureus, Neisseria meningitidis, Haemophilus parainfluenzae, Listeria monocitogenes, Klebsiella pneumonia,* etc.).

The clinical specificity of **AmpliSens® *Streptococcus agalactiae*-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 agalactiae*-screen-titre-FRT** PCR kit for detection and quantitative analysis of *Streptococcus agalactiae* DNA in the clinical material (blood plasma, cerebrospinal fluid (CSF), oropharyngeal swabs, epithelial swabs of lateral vaginal walls, anorectal swabs) using the polymerase chain reaction (PCR) with real-time fluorescence hybridization detection, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

15. QUALITY CONTROL

In compliance with the Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® *Streptococcus agalactiae*-screen-titre-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
13.02.18 PM	3. Content	The colour of the reagent was specified
17.01.19 TA	2. Principle of PCR detection	The information about the enzyme UDG was added. The information about “hot-start” was corrected
13.09.19 PM	Through the text	The text formatting was changed
	9. Data analysis	Results for C- and NCA in the channel for FAM fluorophore were changed from “Absent” to “> boundary value” in Table 4
03.06.20 KK	Footer	The phrase "Not for use in the Russian Federation'' was added
	2. Principle of PCR detection	The table with targets was added
11.03.21 MM	—	The name, address and contact information for Authorized representative in the European Community was changed

AmpliSens®

EC REP

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