AmpliSens® MDR VRE-FRT PCR kit

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

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

1. INTENDED USE

AmpliSens® MDR VRE-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of DNA of *Enterococcus* spp. and *vanA* and *vanB* gene in bacterial culture samples obtained by seeding the biomaterial (tracheal aspirate, bronchoalveolar lavage, blood, cerebrospinal fluid, sputum, urine, traumatic discharge) on liquid or solid medium using real-time hybridization-fluorescence detection of amplified products. Detection of the *vanA* and *vanB* genes is carried out in order to identify strains of *Enterococcus* resistant to the vancomycin antibiotic (VRE). The material for PCR-analysis is DNA samples extracted from test material.

Indications and contra-indications for use of the reagent kit

The reagent kit is used in clinical laboratory diagnostics to study samples of bacterial cultures obtained by inoculation of biomaterial taken from persons with clinical and/or laboratory signs of infections of various localization that can be caused by *Enterococcus*.

There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

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

2. PRINCIPLE OF PCR DETECTION

Principle of testing is based on the DNA extraction from the samples of test material with the exogenous internal control sample (Internal Control (IC)[1]) and simultaneous amplification of DNA fragments of the detected genes (fragments of *Enterococcus* spp. DNA, fragments of enterococcal resistance genes to vancomycin (*vanA* and *vanB*)) and DNA of the internal control with hybridization-fluorescence detection. Exogenous internal control (Internal Control (IC)) allows to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition.

Amplification of DNA/cDNA fragments with the use of specific primers and Taq-polymerase enzyme are performed with the DNA/RNA samples obtained at the extraction stage. 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 PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate (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	Cy5
DNA-target	*vanA* gene	*vanB* gene	*Enterococcus* spp. DNA	Internal Control-FL (IC) DNA
Target gene	fragment of *vanA* gene	fragment of *vanB* gene	fragment of 16S rRNA gene	Artificially synthesized sequence

3. CONTENT

AmpliSens® MDR VRE-FRT PCR kit is produced in 2 forms:

Form 1: BC-express, PCR kit variant FRT-100 F, REF HN-3891-1-CE;

Form 2: BC-express, PCR kit variant FRT-L, REF HN-3892-1-4-CE

BC-express includes:

Reagent	Description	Volume, ml	Quantity
BC-express	colorless clear liquid	5.0	6 tubes

The reagent is intended for extraction of 120 samples (including controls).

Variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-FL VRE	clear liquid from colorless to light lilac colour	1.2	1 tube
PCR-buffer-B	colorless clear liquid	0.6	1 tube
Polymerase (TaqF)	colorless clear liquid	0.06	1 tube
Positive Control-1 VRE	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	1 tube

Variant FRT-100 F is intended for 110 reactions (including controls).

Variant FRT-L includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix MDR VRE-Lyo	white powder	–	96 tubes of 0.2 ml
Positive Control-1 VRE	colorless clear liquid	0.5	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube

Variant FRT-L is intended for 96 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

For sampling and pretreatment

- 0.9 % of sodium chloride (sterile saline solution) or phosphate buffered saline (PBS) (137 mM sodium chloride; 2,7 mM potassium chloride; 10 mM sodium monophosphate; 2 mM potassium diphosphate; pH=7,5±0,2).
- Sterile bacteriological loops.
- Disposable tightly closed polypropylene 1.5 ml tubes for pretreatment.
- Sterile pipette tips with aerosol filters (up to 100, 200 and 1000 µl).
- Tube racks.
- PCR box.
- Pipettes (adjustable).
- Desktop centrifuge up to 12,000 g (suitable for Eppendorf tubes).
- Vacuum aspirator with flask for removing supernatant.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Disposable powder-free gloves and a laboratory coat.
- Reservoir for used tips.

For DNA/RNA extraction, reverse transcription and amplification

- Disposable polypropylene tubes:
  a) screwed or tightly closed 1.5-ml tubes for reaction mixture preparation.
  b) 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;
  c) 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.
- Sterile pipette tips with aerosol filters (up to 100, 200 and 1000 µl).
- Tube racks.
- PCR box.
- Vortex mixer.
- Pipettes (adjustable).
- Real-time instruments (for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 (Bio-Rad, USA)).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Disposable powder-free gloves and a laboratory coat.
- Reservoir for used tips.

[1] Internal Control (IC) is a part of **BC-express** reagent.

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 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 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.
- 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 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

AmpliSens® MDR VRE-FRT PCR kit is intended for analysis of the DNA extracted with BC-express reagent from bacterial culture samples obtained by seeding the biomaterial (tracheal aspirate, bronchoalveolar lavage, blood, cerebrospinal fluid, sputum, urine, traumatic discharge) on liquid or solid medium.

Pretreatment

Pretreatment for bacterial cultures obtained by seeding the biomaterial on solid medium is not required.

It is allowed the preparation of bacterial suspension in PBS-buffer or in 0.9% sodium chloride solution. Transfer 10^7-10^9 of bacterial cells taken by a loop or sterile tip to the tube with 500 µl of PBS-buffer or 0.9% sodium chloride solution. The final suspension is used for further work.

Bacterial cultures obtained by seeding the biomaterial on liquid medium is to be pretreated.

Transfer from 100 to 250 µl of bacterial culture in liquid medium into the sterile disposable 1.5-ml tube (using disposable Pasteur pipette or pipette tip with filter). Centrifuge at 10,000 g (for example, 12,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 10 min. Remove the supernatant using vacuum aspirator with flask without taking the pellet and using the separate tip without filter for each sample. Use the pellet for the DNA extraction.

The bacterial pellet or bacterial suspension can be stored before the PCR analysis:

– at the temperature from minus 24 to minus 16 °C – for 1 week,
– at the temperature not more than minus 68 °C – for a long time.

Interfering substances and limitations of using test material samples

In order to control the DNA extraction efficiency and PCR reaction the Internal Control (Internal Control (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.

Potential interfering substances

To assess the potential interference, samples of bacterial cultures and mixtures of quality control sample (QCS) of the enterprise were tested without the addition and with the addition of 15 mg of agar culture medium (blood agar, LB-agar) or 25 µl of liquid broth (LB) (see Table 2).

For testing, a bacterial culture of *Enterococcus faecium* (containing the *vanA* gene) was used, as well as on a mixture of quality control sample (QCS) containing *Enterococcus* spp. DNA, *vanA* DNA and *vanB* DNA, at a concentration of $5x10^5$ copies/ml of each.

Table 2

Potential interferent	Tested concentration in a sample	Interference presence
Blood sheep agar	15 mg	Not detected
LB-agar	15 mg	Not detected
Liquid broth (LB)	25 µl	Not detected

7. WORKING CONDITIONS

AmpliSens® MDR VRE-FRT PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. DNA extraction

It is recommended to use the following reagent for extraction:

– **BC-express**.

8.1.1 DNA extraction from test samples using BC-express reagent

1. Turn on thermostat and set the temperature to **70 °C**.

When analyzing bacterial culture samples obtained by seeding the biomaterial on solid medium

2. Prepare the required number of empty tubes, including the tube of Negative Control of Extraction (C–), mark them.
3. Transfer **250 µl** of **BC-express**[2] to each tube.
4. Transfer **10^7-10^9** of bacterial cells taken by loop or sterile tip or **20 µl** of bacterial suspension (when analyzing bacterial suspension samples in PBS-buffer or in 0.9% sodium chloride solution) using a separate tip with filter for each sample to the tube with **BC-express.**
5. Do not add anything to the tube of Negative Control of Extraction (C–) except BC-express. See point 8.

When analyzing bacterial culture samples obtained by seeding the biomaterial on liquid medium

6. Transfer **250 µl** of **BC-express** to the tubes with pellet of bacterial cells using a separate tip with filter for each tube.
7. Mark one additional tube as Negative Control of Extraction (C–) and transfer **250 µl** of **BC-express** and **20 µl** of liquid medium. See point 9.
8. Close the tubes and mix by vortex. Sediment the drops by vortex (2-3 sec).
9. Incubate the tubes at **70 °C** for **10 min** in thermostat.
10. Mix and centrifuge the tubes at **12,000 g** for **1 min** (for example, 13,400 rpm for the MiniSpin Eppendorf microcentrifuge). Supernatant contains DNA. The samples are ready for PCR.

DNA samples can be stored at 2–8 °C for 1 week, at the temperature not more than minus 68 °C for 1 year.

NOTE: In case of repeat PCR-analysis of DNA samples it is necessary to mix the tubes by vortex and centrifuge in accordance with point 10.

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

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.

Variant FRT-100 F

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

1. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:
   – **10 µl** of **PCR-mix-FL VRE**,
   – **5 µl** of **PCR-buffer-B**,
   – **0.5 µl** of **polymerase (TaqF)**.

Prepare the reaction mixture for the total number of test and control samples plus one extra reaction. See the number of control samples in item 7.

NOTE: Prepare the reaction mixture just before use.

2. Thaw the tube with **PCR-mix-FL VRE**. Vortex the tubes with **PCR-mix-FL VRE, PCR-buffer-B** and **polymerase (TaqF)**, sediment the drops by vortex.
3. In a new tube prepare the reaction mixture. Mix the required quantities of **PCR-mix-FL VRE**, **PCR-buffer-B** and **polymerase (TaqF)**, 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 reactions:
   C+ – Add **10 µl** of **Positive Control-1 VRE** to the tube with reaction mixture labeled **C+** (Positive Control of Amplification).
   C– – Add **10 µl** of **the sample extracted as C–** to the tube with reaction mixture labeled **C–** (Negative Control of Extraction).
   NCA – Add **10 µl** of **TE-buffer** to the tube with reaction mixture labeled **NCA** (Negative Control of Amplification).

Variant FRT-L

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

1. Take the required number of the tubes with ready-to-use lyophilized reaction mixture **PCR-mix VRE-Lyo** for amplification of DNA from test and control samples (see the number of control samples in item 3).
2. Add **25 µl** of **DNA samples** obtained by extraction.
3. Carry out the control amplification reactions:
   C+ – Add **25 µl** of **Positive Control-1 VRE** to the tube labeled **C+** (Positive Control of Amplification).
   C– – Add **25 µl** of **the sample extracted as C–** to the tube labeled **C–** (Negative control of Extraction).
   NCA – Add **25 µl** of **TE-buffer** to the tube labeled **NCA** (Negative Control of Amplification).

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

8.3.2. Amplification

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

Table 3

AmpliSens-B amplification and detection 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**	**35**	**95**	**5 s**	**35**
	60	**20 s fluorescence acquiring**		**60**	**30 s fluorescence acquiring**	
	72	**15 s**		**72**	**15 s**	

Fluorescent signal is detected in the channels for the **FAM**, **JOE**, **ROX** and **Cy5** fluorophores.

2. Insert tubes into the reaction module of the device. It is recommended to sediment drops from walls of tubes by vortex.

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

3. Run the amplification program with fluorescence detection.
4. Analyze results after the amplification program is completed.

[2] Internal Control (IC) is a part of BC-express reagent.
[3] For example, Rotor-Gene Q (QIAGEN, Germany).
[4] For example, CFX 96 (Bio-Rad, USA).

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time RT-PCR instrument used by measuring fluorescence signal accumulation in two channels:

Table 4

Channel for the fluorophore	FAM	JOE	ROX	Cy5
Amplification product	*vanA* gene	*vanB* gene	*Enterococcus* spp. DNA	Internal Control (IC) DNA

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.
Principle of interpretation is the following:

Table 5

Results interpretation

Ct value in the channel for the fluorophore				Result
FAM	JOE	ROX	Cy5	
absent or > boundary value	absent or > boundary value	absent or > boundary value	< boundary value	*Enterococcus* spp. DNA **is NOT detected** *vanA* and *vanB* genes **are NOT detected**
absent or > boundary value	absent or > boundary value	< boundary value	determined or absent	*Enterococcus* spp. DNA **is detected** *vanA* and *vanB* genes **are NOT detected**
< boundary value	absent or > boundary value	< boundary value	determined or absent	VRE genes **is detected** *vanA* gene **is detected** *vanB* gene **is not detected**
absent or > boundary value	< boundary value	< boundary value	determined or absent	VRE genes **is detected** *vanA* gene **is NOT detected** *vanB* gene **is detected**
< boundary value	< boundary value	< boundary value	determined or absent	VRE genes **is detected** *vanA* gene **is detected** *vanB* gene **is detected**
< boundary value	absent or > boundary value	absent or > boundary value	determined or absent	**Enterococcus* spp. DNA **is NOT detected** *vanA* gene **is detected** *vanB* gene **is NOT detected**
absent or > boundary value	< boundary value	absent or > boundary value	determined or absent	**Enterococcus* spp. DNA **is NOT detected** *vanA* gene **is NOT detected** *vanB* gene **is detected**
< boundary value	< boundary value	absent or > boundary value	determined or absent	**Enterococcus* spp. DNA **is NOT detected** *vanA* gene **is detected** *vanB* gene **is detected**
absent or > boundary value	absent or > boundary value	absent or > boundary value	absent or > boundary value	**Invalid** result**

* This result can be obtained when testing samples of bacterial cultures containing *Staphylococcus* spp., which have the *vanA* and/or *vanB* genes (for example, when testing positive hemoculture).

** In case of **invalid** result, the PCR analysis should be repeated for the corresponding test sample starting beginning with the DNA extraction stage.

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

The result of the analysis is considered reliable only if the results obtained for the controls of amplification and extraction are correct (see Table 6).

Table 6

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore			
		FAM	JOE	ROX	Cy5
C–	DNA extraction	Absent	Absent	Absent	< boundary value
NCA	PCR	Absent	Absent	Absent	Absent
C+	PCR	< boundary value	< boundary value	< boundary value	< boundary value

10. TROUBLESHOOTING

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

1. The *Ct* value determined for the Positive Control of Amplification (C+) in the any channels for the fluorophores (see Table 6) is greater than the boundary *Ct* value or absent. The amplification and detection should be repeated for all samples.
2. The *Ct* value for the Negative Control of Extraction (C–):
   a) is determined in the channel for the FAM and/or JOE and/or ROX 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 PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which specific DNA was detected.
   b) is determined more than boundary value or absent in the channel for the Cy5 fluorophore. This means that the Negative Control of Extraction (C–) did not perform the function of contamination control. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which specific DNA was detected.
3. The *Ct* value is determined for the Negative Control of Amplification (NCA) in the channels for the FAM and/or JOE and/or ROX and/or Cy5 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.
4. 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® MDR VRE-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days. PCR kit can be transported at 2–25 °C for no longer than 3 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® MDR VRE-FRT** PCR kit are to be stored at 2-8 °C when not in use (except for PCR-mix-FL VRE, PCR-buffer-B and polymerase (TaqF)). All components of the **AmpliSens® MDR VRE-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-FL VRE, PCR-buffer-B and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C

NOTE: PCR-mix-FL VRE and PCR-mix VRE-Lyo is to be kept away from light

NOTE: PCR-mix VRE-Lyo is to be kept in packages with a desiccant away from light

13. SPECIFICATIONS

13.1. Analytical sensitivity (limit of detection)

Table 7

Test material	Reagent for DNA extraction	PCR kit	Analytical sensitivity (limit of detection), copies/ml
Bacterial cultures obtained by seeding the biomaterial on liquid or solid[5] medium	BC-express	variant FRT-100 F; variant FRT-L	$5x10^5$

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® MDR VRE-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.
AmpliSens® MDR VRE-FRT PCR kit detects the DNA fragments of claimed microorganisms. The analytical specificity of the PCR kit was confirmed by DNA testing of the following microorganisms:

- strains from ATCC® collection (American Type Culture Collection, USA) in concentration no less than $1x10^7$ GE/ml: *Streptococcus pneumoniae* ATCC® 49619™, *Streptococcus mutans* ATCC® 35668™, *Streptococcus bovis* ATCC® 9809™, *Streptococcus equisimilis* ATCC® 12388™, *Streptococcus agalactiae* ATCC® 13813™, *Streptococcus pyogenes* ATCC® 19615™, *Streptococcus salivarius* ATCC® 13419™, *Streptococcus uberis* ATCC® 700407™, *Staphylococcus aureus* ATCC® 6538P™, *Staphylococcus saprophyticus* ATCC® 49907™, *Staphylococcus epidermidis* ATCC® 12228™, *Staphylococcus haemolyticus* ATCC® 29970™, *Bacteroides fragilis* ATCC® 25285™, *Moraxella catarrhalis* ATCC® 25238™, *Rhodococcus equi* ATCC® 6939™, *Stenotrophomonas maltophilia* ATCC® 13637™, *Pseudomonas aeruginosa* ATCC® 15442™, *Neisseria lactamica* ATCC® 23970™, *Neisseria gonorrhoeae* ATCC® 19424™, *Enterobacter cloacae* ATCC® 13047™, *Enterobacter aerogenes* ATCC® 13048™, *Corynebacterium minutissimum* ATCC® 23348™, *Proteus mirabilis* ATCC® 12453™, *Proteus vulgaris* ATCC® 6380™, *Serratia marcescens* ATCC® 14756™, *Escherichia coli* ATCC® 25922™, *Klebsiella oxytoca* ATCC® 49131™, *Klebsiella pneumoniae* ATCC® 27736™, *Acinetobacter baumannii* ATCC® 19606™, *Candida albicans* ATCC® 14053™, *Candida guilliermondii* ATCC® 6260, *Candida krusei* ATCC® 14243™, *Gardnerella vaginalis* ATCC® 14018™, *Listeria grayi* ATCC® 25401™, *Listeria innocua* ATCC® 33090™, *Listeria monocytogenes* ATCC® 7644™, *Salmonella enterica* ATCC® 14028™.
- human DNA in concentration of 1 mg/ml.

The nonspecific responses were not observed while testing the DNA samples of the above mentioned microorganisms and human DNA.
The clinical specificity of **AmpliSens® MDR VRE-FRT** PCR kit was confirmed in laboratory clinical trials.
The information about interfering substances is specified in the *Interfering substances and limitations of using test material samples.*

13.3. Reproducibility and repeatability

Repeatability and reproducibility were determined by testing positive and negative model samples. Positive samples were a mixture of quality control samples (QCS) containing *Enterococcus* spp. DNA, *vanA* DNA and *vanB* DNA, with concentration of $1x10^6$ copies/ml each, BC-express reagent was used as a negative sample.
Repeatability conditions included testing in the same laboratory, by the same operator, using the same equipment within a short period of time. Reproducibility conditions included testing different lots of PCR kit in different laboratories, by different operators, on different days, using different equipment. The results are presented in Table 8.

Table 8

Sample type	Repeatability		Reproducibility	
	Number of samples	Agreement of results, %	Number of samples	Agreement of results, %
Positive	20	100	40	100
Negative	20	100	40	100

13.4. Diagnostic characteristics

Table 9

The results of testing AmpliSens® MDR VRE-FRT PCR kit in comparison with the reference assay

Samples type	The results of application of AmpliSens® MDR VRE-FRT PCR kit		Results of using the reference assay[6]	
			Positive	Negative
Bacterial cultures obtained by seeding the biomaterial on liquid or solid medium	285 samples were tested	**Positive**	165	0
		Negative	0	120

[5] Bacterial suspension in BC-express reagent of bacterial cultures obtained by seeding on solid medium.
[6] Bacteriological culture and *vanA* and *vanB* gene sequencing were used as a reference assay.

Table 10

Diagnostic characteristics of AmpliSens® MDR VRE-FRT PCR kit

Samples type	Diagnostic sensitivity[7] (with a confidence level of 95 %)	Diagnostic specificity[8] (with a confidence level of 95 %)
Bacterial cultures obtained by seeding the biomaterial on liquid or solid medium	100 (97.8-100) %	100 (97-100) %

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14. REFERENCES

1. Ahmed M. O., Baptiste K. E. Vancomycin-resistant enterococci: a review of antimicrobial resistance mechanisms and perspectives of human and animal health //Microbial Drug Resistance. – 2018. – Т. 24. – №. 5. – С. 590-606.
2. Reyes K., Bardossy A. C., Zervos M. Vancomycin-resistant enterococci: epidemiology, infection prevention, and control // Infectious Disease Clinics. – 2016. – Т. 30. – №. 4. – С. 953-965.

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® MDR VRE-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

AmpliSens®

EC REP

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[7] Relative sensitivity in comparison with applied reference assay.
[8] Relative specificity in comparison with applied reference assay.