



For Professional Use Only

eSens MDR VRE QL PCR kit

REF ES4303A

Instructions for Use

1 INTENDED USE

eSens MDR VRE QL 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)*) 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.

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

Table 1

Channel for fluorophore	FAM	JOE	ROX	Cy5
DNA-target	<i>vanA gene</i>	<i>vanB gene</i>	<i>Enterococcus</i> spp. DNA	Internal Control-FL (IC) DNA
Target gene	fragment of <i>vanA gene</i>	fragment of <i>vanB gene</i>	fragment of 16S rRNA gene	Artificially synthesized sequence

3 CONTENT

eSens MDR VRE QL PCR kit (ES4303A) 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).

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

eSens MDR VRE QL PCR kit is intended for 110 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 PCR 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 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- 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.

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

eSens MDR VRE QL 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

6.1 Bacterial cultures

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 5×10^5 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

eSens MDR VRE QL 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

Any commercial nucleic acid extraction kit, if IVD-CE validated for the indicated specimen types, could be used.

Ecoli Dx, s.r.o. recommends:

- For the manual 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*** to each tube.
4. Transfer **10⁷-10⁹** 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.

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

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.

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).

8.2.2 Amplification

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

Table 3

eSens-B amplification and detection program

	Rotor-type instruments (e.g Rotor-Gene Q or equivalent)			Plate-type instruments (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent.)		
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.

8.3 Instrument Settings

Test settings for rotor-type instruments

Channel	Calibrate/Gain Optimisation	Threshold	Dynamic tube	Slope Correct	More Settings/ Outlier Removal
FAM/Green	from 5FI to 10FI	0.1	on	on	5-15 %
JOE/Yellow	from 5FI to 10FI	0.1	on	on	5-15 %
ROX/Orange	from 5FI to 10FI	0.1	on	on	5-15 %
Cy5/Red	from 5FI to 10FI	0.1	on	on	5-15 %

Note: Select the **Reaction volume-25 µl**. Tick the **15 µl oil layer volume**, if this option is provided in the instrument software.

Test settings for plate-type instruments

Note: Set **Ramp Rate 2,5 °C/s** by clicking the **Step Options** button for each step of cycling.

Channel	Threshold
FAM, HEX, ROX, Cy5	Set the threshold line at the level corresponding to 10-20 % of maximum fluorescence level obtained for C+ sample at the last amplification cycle.

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	<i>vanA</i> gene	<i>vanB</i> gene	<i>Enterococcus</i> 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	<i>Enterococcus</i> spp. DNA is NOT detected <i>vanA</i> and <i>vanB</i> genes are NOT detected
absent or > boundary value	absent or > boundary value	< boundary value	determined or absent	<i>Enterococcus</i> spp. DNA is detected <i>vanA</i> and <i>vanB</i> genes are NOT detected
< boundary value	absent or > boundary value	< boundary value	determined or absent	VRE genes is detected <i>vanA</i> gene is detected <i>vanB</i> gene is not detected
absent or > boundary value	< boundary value	< boundary value	determined or absent	VRE genes is detected <i>vanA</i> gene is NOT detected <i>vanB</i> gene is detected
< boundary value	< boundary value	< boundary value	determined or absent	VRE genes is detected <i>vanA</i> gene is detected <i>vanB</i> gene is detected
< boundary value	absent or > boundary value	absent or > boundary value	determined or absent	* <i>Enterococcus</i> spp. DNA is NOT detected <i>vanA</i> gene is detected <i>vanB</i> gene is NOT detected
absent or > boundary value	< boundary value	absent or > boundary value	determined or absent	* <i>Enterococcus</i> spp. DNA is NOT detected <i>vanA</i> gene is NOT detected <i>vanB</i> gene is detected
< boundary value	< boundary value	absent or > boundary value	determined or absent	* <i>Enterococcus</i> spp. DNA is NOT detected <i>vanA</i> gene is detected <i>vanB</i> 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.

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 and Table 7).

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

Table 7

Boundary Ct values

Sample	Rotor-type instruments				Plate-type instruments			
	Channel for fluorophore							
	FAM	JOE	ROX	Cy5	FAM	JOE	ROX	Cy5
C+	30	30	31	30	31	31	32	31
NCA	Ct is absent				Ct is absent			
C-	Ct is absent			34	Ct is absent			34
Test sample	33	33	33	34	34	34	34	35

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.

- 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.

11 TRANSPORTATION

eSens MDR VRE QL 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 **eSens MDR VRE QL 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 **eSens MDR VRE QL 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 is to be kept away from light

13 SPECIFICATIONS

13.1 Analytical sensitivity (limit of detection)

Table 8

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* medium	BC-express	ES4303A	5x10 ⁵

* Bacterial suspension in BC-express reagent of bacterial cultures obtained by seeding on solid medium.

13.2 Analytical specificity

The analytical specificity of **eSens MDR VRE QL 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.

eSens MDR VRE QL 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 1x10⁷ GE/ml: *Streptococcus pneumoniae* ATCC 49619™, *Streptococcus mutans* ATCC

35668TM, *Streptococcus bovis* ATCC 9809TM, *Streptococcus equisimilis* ATCC 12388TM, *Streptococcus agalactiae* ATCC 13813TM, *Streptococcus pyogenes* ATCC 19615TM, *Streptococcus salivarius* ATCC 13419TM, *Streptococcus uberis* ATCC 700407TM, *Staphylococcus aureus* ATCC 6538PTM, *Staphylococcus saprophyticus* ATCC 49907TM, *Staphylococcus epidermidis* ATCC 12228TM, *Staphylococcus haemolyticus* ATCC 29970TM, *Bacteroides fragilis* ATCC 25285TM, *Moraxella catarrhalis* ATCC 25238TM, *Rhodococcus equi* ATCC 6939TM, *Stenotrophomonas maltophilia* ATCC 13637TM, *Pseudomonas aeruginosa* ATCC 15442TM, *Neisseria lactamica* ATCC 23970TM, *Neisseria gonorrhoeae* ATCC 19424TM, *Enterobacter cloacae* ATCC 13047TM, *Enterobacter aerogenes* ATCC 13048TM, *Corynebacterium minutissimum* ATCC 23348TM, *Proteus mirabilis* ATCC 12453TM, *Proteus vulgaris* ATCC 6380TM, *Serratia marcescens* ATCC 14756TM, *Escherichia coli* ATCC 25922TM, *Klebsiella oxytoca* ATCC 49131TM, *Klebsiella pneumoniae* ATCC 27736TM, *Acinetobacter baumannii* ATCC 19606TM, *Candida albicans* ATCC 14053TM, *Candida guilliermondii* ATCC 6260, *Candida krusei* ATCC 14243TM, *Gardnerella vaginalis* ATCC 14018TM, *Listeria grayi* ATCC 25401TM, *Listeria innocua* ATCC 33090TM, *Listeria monocytogenes* ATCC 7644TM, *Salmonella enterica* ATCC 14028TM.

- 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 **eSens MDR VRE QL 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 1x10⁶ 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 9.

Table 9

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 10

The results of testing eSens MDR VRE QL PCR kit in comparison with the reference assay

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

* Bacteriological culture and *vanA* and *vanB* gene sequencing were used as a reference assay.

Table 11

Diagnostic characteristics of eSens MDR VRE QL PCR kit

Samples type	Diagnostic sensitivity* (with a confidence level of 95 %)	Diagnostic specificity** (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) %

* Relative sensitivity in comparison with applied reference assay.

** Relative specificity in comparison with applied reference assay.

14 QUALITY CONTROL

The production process, including batch release, is carried out in accordance with an established quality management system certified according to ISO 13485.

15 KEY TO SYMBOLS USED

 REF	Catalogue number		Keep dry
 LOT	Batch code		Caution
 IVD	<i>In vitro</i> 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

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

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