ECOLI

eSens

CE

IVD

For Professional Use Only

# eSens MDR MCR-1 QL PCR kit

**REF ES4304A**

## Instructions for Use

## 1 INTENDED USE

**eSens MDR MCR-1 QL PCR kit** is an *in vitro* nucleic acid amplification test for qualitative detection of plasmid-encoded *mcr*-1 colistin resistance genes and marker genes of enterobacteria (*Enterobacterales* order) (16S rRNA genes) in bacterial culture samples obtained by seeding the biomaterial (cerebrospinal fluid (CSF), punctate from lesions of organs and tissues, traumatic discharge, tracheal aspirate, bronchoalveolar lavage, blood, sputum, urine, flushing from medical equipment, tools and inventory, wash or food homogenate) on liquid or solid medium using real-time hybridization-fluorescence detection of amplified products. Detection of *mcr*-1 group genes is carried out in order to identify *Enterobacterales* strains resistant to the colistin. 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 infections caused by bacteria of the *Enterobacterales* order, regardless of the form and presence of the manifestation of the disease, as well as for the study of environmental objects and food.

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 together with the exogenous internal control sample (Internal Control (IC)*) and simultaneous amplification of DNA fragments of the detected genes (fragments of *Enterobacterales* DNA (16S rRNA genes) 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 fragments is carried out using primers specific to this region and the Taq-polymerase enzyme. 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 amplification of three DNA targets is carried out in one tube at the stage of amplification.

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** |
|---|---|---|---|
| DNA-target | *Enterobacterales* DNA | *mcr*-1 genes | IC DNA |
| Target gene | fragment of 16S rRNA gene | fragment of *mcr*-1 genes | Artificially synthesized sequence |

# 3 CONTENT

**eSens MDR MCR-1 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 110 samples (including controls).

| **Reagent** | **Description** | **Volume, ml** | **Quantity** |
|---|---|---|---|
| **PCR-mix-FL *MCR*-1** | 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 *MCR*-1** | colorless clear liquid | 0.2 | 1 tube |
| **TE-buffer** | colorless clear liquid | 0.2 | 1 tube |

**eSens MDR MCR-1 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 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 10, 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 MCR-1 QL PCR kit** is intended for analysis of the DNA extracted with BC-express reagent from bacterial culture samples obtained by seeding the biomaterial (cerebrospinal fluid (CSF), punctate from lesions of organs and tissues, traumatic discharge, tracheal aspirate, bronchoalveolar lavage, blood, cerebrospinal fluid, sputum, urine, flushing from medical equipment, tools and inventory, flushing or homogenate of food) 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 potentially interfering substances (see Table 2).
The model samples contained quality control sample (QCS) containing *Enterobacterales* DNA at least at a concentration of $1x10^4$ GE/ml of each as well as a model bacterial culture containing *mcr*-1 genes at a concentration of at least $1x10^7$ CFU/ml.

**Table 2**

| Potential interferent | Tested concentration in a sample | Interference presence |
|---|---|---|
| Agarized nutrient medium 'Endo' | 15 mg | Detected |
| Agarized nutrient medium 'Uriselect' | 15 mg | Detected |
| Liquid nutrient medium 'Endo' | 20 µl | Not detected |
| Liquid nutrient medium 'Uriselect' | 20 µl | Not detected |
| Blood agar | 15 mgl | Not detected |

# 7 WORKING CONDITIONS

**eSens MDR MCR-1 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. To the tube with **BC-express** 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**.**

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 MCR-1,**
   **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 MCR-1**. Vortex the tubes with **PCR-mix-FL MCR-1, 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 MCR-1, 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 MCR-1** 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** and **ROX** 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.

# 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** |
|---|---|---|---|
| Amplification product | *Enterobacterales* DNA | *mcr-1* genes | 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** | |
| absent or > boundary value | absent or > boundary value | < boundary value | *Enterobacterales* DNA **is NOT detected** *mcr-1* genes **are NOT detected** |
| < boundary value | absent or > boundary value | determined or absent | *Enterobacterales* DNA **is detected** *mcr-1* genes **are not detected** |
| < boundary value | < boundary value | determined or absent | *Enterobacterales* **DNA is detected** *mcr-1* genes **are detected** |
| absent or > boundary value | < boundary value | determined or absent | *Enterobacterales* D**NA is NOT detected** *mcr-1* genes **are detected*** |
| 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 *Enterobacterales*., which have the *mcr-1* genes for example *Acinetobacter baumannii* bacteria), 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)**

**Table 6**

**Results for controls**

| **Control** | **Stage for control** | ***Ct* value in the channel for fluorophore** | | |
|---|---|---|---|---|
| | | **FAM** | **JOE** | **ROX** |
| C– | DNA extraction | Absent | Absent | < 32 |
| NCA | PCR | Absent | Absent | Absent |
| C+ | PCR | < 32 | < 32 | < 32 |

# 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 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 ROX 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 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.

# 11 TRANSPORTATION

**eSens MDR MCR-1 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 MCR-1 QL PCR kit** are to be stored at 2–8 °C when not in use (except for PCR-mix-FL MCR-1, PCR-buffer-B and polymerase (TaqF)). All components of the **eSens MDR MCR-1 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 MCR-1, PCR-buffer-B and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C

**NOTE:** PCR-mix-FL MCR-1 is to be kept 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* 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 MCR-1 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 MCR-1 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 $1x10^7$ GE/ml: *Streptococcus pneumoniae* ATCC 49619™, *Streptococcus mutans* ATCC 35668™, *Streptococcus bovis (Group D)* 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 (Branhamella) catarrhalis* ATCC 25238™, *Rhodococcus equi* ATCC 6939™, *Stenotrophomonas maltophilia* ATCC 13637™, *Staphylococcus aureus subsp. aureus* ATCC 12600™, *Neisseria lactamica* ATCC 23970™, *Enterobacter cloacae* ATCC 13047™, *Enterobacter aerogenes* ATCC 13048™, *Corynebacterium jeikeium* ATCC 43734™, *Corynebacterium xerosis* ATCC 373™, *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™, *Listeria grayi (murrayi)* ATCC 25401™, *Listeria innocua* ATCC 33090™, *Listeria monocytogenes* ATCC 7644™.
- 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 MCR-1 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 *mcr*-1 gene, with

concentration of $1x10^6$ copies/ml and *Enterobacterales* DNA, with concentration of $5x10^5$ copies/ml; 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 | 10 | 100 | 40 | 100 |
| Negative | 10 | 100 | 40 | 100 |

## 13.4 Diagnostic characteristics

261 samples of pure culture obtained by seeding the biomaterial on mediums were used for determination of diagnostic characteristics.

Bacteriological seeding with subsequent species-level identification of microorganisms by MALDI-TOF mass spectrometry as well as direct sequencing were used as a reference assay.

**Table 9**

**The results of testing eSens MDR MCR-1 QL PCR kit in comparison with the reference assay**

| Samples type | The results of application of eSens MDR MCR-1 QL PCR kit | | Results of using the reference assay | |
|---|---|---|---|---|
| | | | Positive | Negative |
| Bacterial cultures obtained by seeding the biomaterial on liquid or solid medium | 261 samples were tested | **Positive** | 108 | 0 |
| | | **Negative** | 0 | 153 |

**Table 10**

**Diagnostic characteristics of eSens MDR MCR-1 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 (96.6-100) % | 100 (97.6-100) % |

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