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For Professional Use Only

AmpliSens® *MRSA*-screen-titre-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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TABLE OF CONTENTS

1. INTENDED USE

AmpliSens® *MRSA*-screen-titre-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and quantification of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* DNA and methicillin-resistant coagulase-negative *Staphylococcus* spp. DNA in the biological materials (oropharyngeal swabs, bronchoalveolar lavage (BAL), sputum, endotracheal aspirate, bronchial washes, urine pellet, blood, blood plasma, cerebrospinal fluid (CSF), puncture samples from affected organs and tissues, washes from healthcare equipment and instruments) by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

Detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. In real-time PCR, the amplified product is detected using 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.

AmpliSens® *MRSA*-screen-titre-FRT PCR contains the Internal Control (Internal Control STI-87). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

AmpliSens® *MRSA*-screen-titre-FRT PCR kit uses “hot-start,” which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min

3. CONTENT

AmpliSens® *MRSA*-screen-titre-FRT PCR kit is produced in 2 forms:

AmpliSens® *MRSA*-screen-titre-FRT PCR kit variant FRT-100 F (for use with RG, iQ) **REF** R-B78-100-FT(RG,iQ)-CE.

AmpliSens® *MRSA*-screen-titre-FRT PCR kit variant FRT-100 F in bulk[1].

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

[1] In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FRT *MRSA*	colorless clear liquid	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.6	1 tube
Polymerase (TaqF)	colorless clear liquid	0.06	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
DNA calibrator К1 *MRSA*	colorless clear liquid	0.2	1 tube
DNA calibrator К2 *MRSA*	colorless clear liquid	0.2	1 tube
Negative Control (С–)*	colorless clear liquid	1.2	2 tubes
Positive Control DNA *MRSA* (С+$_{MRSA}$)**	colorless clear liquid	0.1	1 tube
Internal Control STI-87 (IC)***	colorless clear liquid	0.6	2 tubes

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

** must be used in the extraction procedure as Positive Control of Extraction (PCE).

*** add 10 µl of Internal Control STI-87 during the DNA extraction procedure directly to the sample/lysis mixture (see RIBO-prep **REF** K2-9-Et-100-CE).

AmpliSens® *MRSA*-screen-titre-FRT PCR kit is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit or the DNA extraction automatic station.
- Disposable powder-free gloves and laboratory coat.
- Automatic adjustable pipettes (from 5 to 20 µl and from 20 to 200 µl).
- Disposable tips with aerosol filters (up to 100 µl) in tube racks.
- Tube racks.
- Vortex mixer/desktop centrifuge.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); iCycler iQ, iCycler iQ5 (Bio-Rad, USA)).
- Disposable polypropylene tubes for PCR of 0.2- or 0.1-ml:
    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 at 2 to 8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir bin 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 (samples, 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 afterward.
- 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 samples and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance 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 samples and reagents contact with the skin, eyes and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the 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 to 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

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® *MRSA*-screen-titre-FRT PCR kit is intended for the analysis of DNA extracted from oropharyngeal swabs, BAL, sputum, endotracheal aspirate, bronchial washes, urine pellet, blood, blood plasma, CSF, puncture samples from affected organs and tissues, washes from healthcare equipment and instruments.

- *Blood plasma*.

Tubes with collected whole blood are centrifuged at 800 g for 10 min at room temperature. Blood plasma samples of 1.0 ml volume are transferred to sterile tubes (for example, 1.5-2.0 ml Eppendorf tubes). Use tips with aerosol barriers. These tubes are centrifuged at 11,000 rpm for 10-20 min. DNA should be extracted from the pellet together with 100 µl of supernatant.

7. WORKING CONDITIONS

AmpliSens® *MRSA*-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 kit:

- RIBO-prep, **REF** K2-9-Et-100-CE.
- NucliSENS easyMAG automated system can also be used.

Extract DNA according to the manufacturer's protocol.

If extracting with RIBO-prep reagent kit pay attention to the following:

- DNA is extracted in the presence of the internal control sample: add **10 µl** of **Internal Control STI-87 (IC)** to each sample.
- to the tube labeled **C–** (**Negative Control of extraction)** transfer 100 µl of Negative Control (C–) reagent;
- to the tube labeled **PCE** (**Positive Control of extraction)** transfer 90 µl of Negative Control (C–) and 10 µl of Positive Control DNA *MRSA* ($C+_{MRSA}$).

DNA extraction with NucliSENS easyMAG automated system is described in Guidelines [2].

8.2. Preparing PCR

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

8.2.1 Preparing tubes for PCR

1. Prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**. To do this, transfer the entire content of the tube with **polymerase (TaqF) (60 µl)** into the tube with **PCR-mix-2-FRT (600 µl)** and carefully vortex. Avoid foaming. Centrifuge tubes for 1-2 s to remove drops from tube walls. Indicate the date of mixture preparation on the tube.

The prepared mixture is intended for 120 samples.
Store at 2–8 °C for 3 months and use as needed.

If the mixture volume will not be utilized within 3 months it is necessary to prepare mixture for less number of reactions. For example, mix **150 µl** of **PCR-mix-2-FRT** and **15 µl** of **polymerase (TaqF)**. The obtained mixture is intended for 30 reactions.

2. Prepare the reaction mixture. Note that for analysis of even one clinical sample it is necessary to run five controls of amplification stage: two DNA calibrators (**К1 *MRSA*** and **К2 *MRSA***) in two repeats and the Negative Control of amplification (DNA-buffer). In addition, include one extra reaction when calculating reagent volumes: for detection of N samples take the reagents for N+1 reactions.
3. Mix **PCR-mix-1-FRT *MRSA*** and the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)** in a new tube in the following proportion:
   - **10 µl** of **PCR-mix-1-FRT *MRSA*,**
   - **5 µl** of the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF).**

One can calculate reagent volume for the needed number of reactions according to the scheme given in the Table 1.

Table 1

Scheme of reaction mixture preparation

	Reaction volume	
Reagent volume for 1 reaction (µl)	**10.0**	**5.0**
Number of biological samples	**PCR-mix-1-FRT *MRSA*[2]**	**Mixture of PCR-mix-2-FRT and polymerase (TaqF)**
1	**70**	**35**
2	**80**	**40**
3	**90**	**45**

[2] Values are given with account of one extra reaction and five controls of amplification stage: 2 DNA calibrators, К1 MRSA and К2 MRSA, (in two replicates) and the Negative Control (DNA-buffer)

4	**100**	**50**
5	**110**	**55**
6	**120**	**60**
7	**130**	**65**
8	**140**	**70**
9	**150**	**75**
10	**160**	**80**
11	**170**	**85**
12	**180**	**90**
13	**190**	**95**
14	**200**	**100**
15	**210**	**105**
16	**220**	**110**
17	**230**	**115**
18	**240**	**120**
19	**250**	**125**
20	**260**	**130**
21	**270**	**135**
22	**280**	**140**
23	**290**	**145**
24	**300**	**150**
25	**310**	**155**
30	**360**	**180**

4. Take the required quantity of tubes for amplification of clinical and control DNA samples.
5. Transfer **15 µl** of the prepared mixture to each tube.
6. Add **10 µl** of **DNA** obtained from clinical or control samples to the tubes with the reaction mixture.
7. Prepare control reaction:

NCA - add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of amplification).

K1 *MRSA* - add **10 µl** of **K1 *MRSA*** to two tubes and **10 µl** of **K2 *MRSA*** to the other two tubes.
K2 *MRSA*

C– - add **10 µl** of the sample extracted from the **Negative Control (C–)** reagent (Negative Control of extraction)

PCE - add **10 µl** of **DNA** extracted from the **Positive Control DNA *MRSA* (C+$_{MRSA}$)** (Positive Control of extraction).

8.2. 2. Amplification

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

Table 2a

MRSA amplification program for rotor-type instruments[3]

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*
Hold	95	15 min	–	1
Cycling 1	95	15 s	–	5
	60	30 s	–	
	72	15 s	–	
Cycling 2	95	15 s	–	40
	55	30 s	FAM/Green, JOE/Yellow, ROX/Orange	
	72	15 s	–	

Table 2b

MRSA amplification program for plate-type instruments[4]

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*
1	95	15 min	–	1
2	95	15 s	–	5
	55	30 s	–	
	72	15 s	–	
3	95	15 s	–	40
	55	30 s	FAM, HEX/JOE, ROX	
	72	15 s	–	

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.

9. DATA ANALYSIS

Analysis of results is carried out with the software of the used real-time PCR instrument. Curves of fluorescence signal accumulation are analyzed in three channels:

- the amplification product of the *Staphylococcus aureus* DNA fragment is detected in the channel for the FAM fluorophore,
- the amplification product of the *mecA* gene fragment, which is located in the chromosome of *S.aureus* and some other *Staphylococcus* species in the specific region found in the methicillin-resistant strains only, is detected in the channel for the JOE fluorophore,
- the Internal Control STI-87 (IC) DNA is detected in the channel for the ROX fluorophore.

Interpretation of results

The results are interpreted by the software of the used real-time PCR instrument by the

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

crossing (or not crossing) of the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of the *Ct* (cycle threshold) value in the results grid. The principle of interpretation is outlined in table 3.

Table 3

Interpretation of results and quantification for clinical samples

Channel for the fluorophore			**Result (calculation of concentration, copies/ml)**
FAM (*S.aureus*)	**JOE (*mecA* gene)**	**ROX (IC)**	
+	**–**	**+/–**	***MSSA* (= (A/C)*IC coefficient*N)**
–	**+**	**+/–**	***MRCoNS* (= (B/C)* IC coefficient*N)**
+	**+**	**+/–**	**Calculate log A and log B.** **1)** If **A** differs from **B** by not more than **0.3 log,** the result is ***MRSA* (= (B/C)* IC coefficient*N)** **2)** If **A** differs from **B** by more than **0.3 log,** the result is ***MRSspp.(MRSA* and *MRCoNS)* (= (B/C)* IC coefficient*N)**
–	**–**	**–**	**Invalid**
–	**–**	**+**	**Not detected**

where **A** – calculated concentration in the channel for the FAM fluorophore;

B – calculated concentration in the channel for the JOE fluorophore;

C – calculated concentration in the channel for the ROX fluorophore;

$$\mathbf{N=\frac{100}{extraction\ volume,\ \mu l}}$$

IC coefficient is specified in the *Important Product Information Bulletin* and cannot be used for calculation of results obtained with the reagents of different lot.

– **DNA of *MSSA* (methicillin-sensitive *Staphylococcus aureus*) is detected** if a *Ct* value is defined in the the channel for the FAM fluorophore and there is no *Ct* value in the channel for the JOE fluorophore (the fluorescence curve does not cross the threshold line).

Concentration is calculated as follows:

(A/C)*IC coefficient*N = (copies/ml of sample)

– **DNA of *MRCoNS* (methicillin-resistant coagulase-negative *Staphylococcus* spp.) is detected** if a *Ct* value in the channel for the JOE fluorophore is defined and there is no *Ct* value in the channel for the FAM fluorophore (fluorescence curve does not cross the threshold line).

Concentration is calculated as follows:

(B/C)*IC coefficient*N = (copies/ml of a sample)

– **DNA of *MRSA* (methicillin-resistant *Staphylococcus aureus*) is detected** in a sample if the *Ct* value is defined in the channels for the FAM and JOE fluorophores. Moreover, the fluorescence curves should cross the threshold line at the area of exponential growth of fluorescence and the difference between logarithms of calculated concentrations in the channels for the FAM and JOE fluorophores is not more than **0.3** (see table 2). Concentration is calculated as follows:

(B/C)*IC coefficient*N = (copies/ml of sample)

If the common logarithm of calculated concentration in the channel for the FAM fluorophore differs from that in the channel for the JOE fluorophore by more than **0.3**, then the displayed result is ***"DNA of MRSspp. (MRSA and MRCoNS) (methicillin-resistant Staphylococcus spp. including methicillin-resistant Staphylococcus aureus and methicillin-resistant coagulase-negative Staphylococcus spp.)"***

Concentration is calculated as follows:

(B/C)*IC coefficient*N = (copies/ml of sample)

Result is considered **invalid** if a *Ct* value is not defined in the results grid in the channel for the FAM fluorophore, whereas the *Ct* value in channels for the JOE and ROX channels is absent or the calculated value is less than the value specified in the *Important Product Information Bulletin*. The PCR analysis should be repeated again for such samples.

Linear measuring range of AmpliSens® *MRSA*-screen-titre-FRT PCR kit is 800–10,000,000 copies/ml. If the result is greater than 10,000,000 copies/ml, it is indicated as ***the result is greater than 10,000,000 copies/ml***. If the result is less than 800 copies/ml, it is indicated as ***the result is less than 800 copies/ml***.

Concentration values of DNA calibrators are specified in *Important Product Information Bulletin*

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

Table 4

Results for controls

Control	Stage for control	Result of amplification in the channel for the fluorophore		
		FAM	JOE	ROX
C–	DNA extraction	Absent	Absent	> boundary value

PCE	DNA extraction, PCR	Value is within the range	Value is within the range	> boundary value
NCA	PCR	Absent	Absent	Absent
K1 *MRSA* **K2 *MRSA***	PCR	*Ct* value and calculated concentration are determined	*Ct* value and calculated concentration are determined	*Ct* value and calculated concentration are determined

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

10. TROUBLESHOOTING

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

1. If an **invalid** result is obtained it is necessary to repeat PCR analysis of the required biological sample.
2. Absence of positive signal in DNA calibrators can result from incorrect settings of amplification program or failures of PCR preparation. In that case it is necessary to repeat PCR again for all samples.
3. If a positive signal is detected for the Negative Control of extraction (C–) in the FAM and/or JOE channels and for the Negative Control of amplification (NCA) in any of the channels, FAM, JOE and/or ROX, it means that contamination of reagents or samples has occurred. In that case results for all samples are considered to be invalid. The analysis must be repeated and measures for detecting and eliminating the contamination source must be taken;
4. If a positive result is detected for a test sample, whereas its fluorescent curve does not have exponential slope (it more looks like straight line), it means that the threshold or baseline parameters are set incorrectly. This result can't be considered as positive. If the threshold value was correct it is necessary to repeat PCR for this sample.

11. TRANSPORTATION

AmpliSens® *MRSA*-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® *MRSA*-screen-titre-FRT** PCR kit (except for PCR-mix-1-FRT *MRSA*, PCR-mix-2-FRT, and polymerase (TaqF)) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *MRSA*-screen-titre-FRT** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is

the same, unless otherwise stated.

PCR-mix-1-FRT *MRSA*, PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at temperature from minus 24 to minus 16 °C when not in use.

PCR-mix-1-FRT *MRSA* is to be kept away from light.

13. SPECIFICATIONS

13.1 Sensitivity

Test material	Nucleic acid extraction kit	Analytical sensitivity, copies/ml	Linear measuring range, copies/ml
– oropharyngeal swabs, – BAL, – sputum, – endotracheal aspirate, – bronchial washes, – urine[5], – blood, blood plasma, – CSF, – puncture samples from affected organs and tissues, – washes from healthcare equipment and instruments	RIBO-prep	400	800 – 10,000,000

13.2 Specificity

The analytical specificity of **AmpliSens® *MRSA*-screen-titre-FRT** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis.

The specific activity of **AmpliSens® *MRSA*-screen-titre-FRT** PCR kit was confirmed in studies of bacterial strains of *Staphylococcus aureus* including *MRSA*, as well as by analyzing clinical material with subsequent confirmation of results by sequencing the amplified fragments.

Analytical specificity was tested on the following strains and isolates: *Chlamydophila pneumonia*, *Escherichia coli*, *Haemophilus haemolyticus*, *H.influenzae*, *H.parainfluenzae*, *Klebsiella oxytoca*, *K.pneumonia*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium tuberculosis*, *Mycoplasma pneumonia*, *Neisseria cinereae*, *N.elongata*, *N.flavescens*, *N.gonorrhoeae*, *N. meningitidis*, *N.mucosa*, *N.sicca*, *N.subflava*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella flexneri*,

[5] Pretreatment is required.

Streptococcus agalactiae, *S.milleri*, *S.mitis*, *S.mutans*, *S. pneumoniae*, *S.pyogenes*, *S.salivarius*, *S.sanguis*, *S.suis*, *S.viridans*, as well as human genome DNA. Testing the above-mentioned strains with this PCR kit did not reveal nonspecific responses.

The clinical specificity of **AmpliSens® *MRSA*-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, 2008.
2. Guidelines to the AmpliSens® *MRSA*-screen-titre-FRT PCR kit for qualitative detection and quantification of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* DNA and methicillin-resistant coagulase-negative *Staphylococcus* spp. DNA in the biological materials by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

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® *MRSA*-screen-titre-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
RUO	Research use only		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
MRSA	Methicillin-resistant *Staphylococcus aureus*	**C+**	Positive control of amplification
MSSA	Methicillin-sensitive *Staphylococcus aureus*	**PCE**	Positive Control of Extraction
MRCoNS	Methicillin-resistant coagulase-negative *Staphylococcus* spp.	**K1 *MRSA*, K2 *MRSA***	DNA Calibrators
		MRSspp.	Methicillin-resistant *Staphylococcus* spp.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
20.04.12 Ivl	Title page, Key to symbols used	Symbol IVD in vitro diagnostic medical device was changed to RUO research use only
16.07.12 LA	1. Intended Use	Intended use was corrected «…methicillin-sensitive and methicillin-resistant Staphylococcus aureus DNA and methicillin-resistant coagulase-negative Staphylococcus spp. DNA in the biological materials (oropharyngeal swabs, bronchoalveolar lavage, sputum, endotracheal aspirate, bronchial washes, urine pellet, blood, blood plasma, cerebrospinal fluid (CSF), puncture samples from affected organs and tissues, washes from healthcare equipment and instruments)…» instead of «… methicillin-resistant Staphylococcus aureus (MRSA) DNA in the clinical materials (oropharyngeal swabs, bronchoalveolar lavage, sputum, urine pellet, blood plasma) and environmental samples (taken from healthcare instruments) …»
	2. Principle of PCR Detection	The following phrase was deleted: “The PCR kit is to detect the fragment of Staphylococcus aureus DNA and the fragment of mecA gene located in the specific area found only in methicillin-resistant strains of staphylococci”
	6. Sampling and Handling 13. Specification	The following biological material was added: endotracheal aspirate, bronchial washes, blood, cerebrospinal fluid (CSF), puncture samples from affected organs and tissues, washes from healthcare equipment and instruments
	9. Data Analysis	Information if the “Result” column of Table 2. Interpretation of results and quantification for clinical samples was corrected
		Interpretation of results and formula for calculation of concentration are added for DNA of MRSspp.
	13.2 Specificity	Information was corrected
	16. Key To Symbols Used	MRSspp. (Methicillin-resistant Staphylococcus spp.) was added
		MRCoSA (Methicillin-resistant coagulase-negative Staphylococci) was corrected to MRCoNS (Methicillin-resistant coagulase-negative Staphylococcus spp.)
25.02.14 GA	3. Content	In bulk form was added
	4. Additional requirements	Chapter was corrected accordance to the pattern
	8. Protocol	*Scheme of reaction mixture preparation* was added
	Footer	Catalogue number **REF** R-B78-100-FT(RG,iQ)-CE-B was added