AmpliSens® *Mycoplasma pneumoniae / Chlamydophila pneumoniae*-FRT PCR kit

CE 1023

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

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

1. INTENDED USE

AmpliSens® *Mycoplasma pneumoniae / Chlamydophila pneumoniae*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of the DNA of *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* in the biological material (sputum (or tracheal aspirate), bronchial washing fluid, bronchoalveolar lavage, nasopharyngeal and oropharyngeal swabs, and autopsy material) using real-time hybridization-fluorescence detection.

The PCR kit is also used for studying the role of *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* role in the pathogenesis of noninfectious chronic diseases, such as cardiovascular system diseases, by the *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* DNA detection in the whole blood by using nucleic acid extraction kit RIBO-sorb.

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

2. PRINCIPLE OF PCR DETECTION

Mycoplasma pneumoniae and *Chlamydophila pneumoniae* detection by the polymerase chain reaction (PCR) is based on the amplification of a pathogen genome specific regions (*putative lipoprotein* of *Mycoplasma pneumoniae* and *ompA* of *Chlamydophila pneumonia)* 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 the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® *Mycoplasma pneumoniae / Chlamydophila pneumoniae*-FRT PCR kit is a qualitative test that uses the principle of endogenous control (amplification of human prothrombin gene fragment). It allows to control the presence of cells and human DNA in a sample in a sufficient quantity. Thus, the use of an endogenous internal control makes it possible not only to monitor test stages (DNA extraction and amplification) but also to assess the adequacy of sampling and storage of clinical material.

AmpliSens® *Mycoplasma pneumoniae / Chlamydophila pneumoniae*-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. In variant FRT-100 F, "hot-start" is guaranteed by the separation of nucleotides and Taq-polymerase by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate. 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 deoxyuridine triphosphate is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

The results of amplification are registered in the following fluorescence channels.

Table 1

Channel for fluorophore	FAM	JOE	ROX
DNA-target	*Mycoplasma pneumoniae*	Internal Control – human DNA	*Chlamydophila pneumoniae*
Target gene	Putative lipoprotein	Human protrombin gene V	ompA

3. CONTENT

AmpliSens® *Mycoplasma pneumoniae / Chlamydophila pneumoniae*-FRT PCR kit is produced in 1 form:

variant FRT-100 F REF R-B42-100-F-CE.

Variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT (F) *Mycoplasma pneumoniae / Chlamydophila pneumoniae*	colorless clear liquid	0.2	5 tubes
PCR-mix-2-FRT	colorless clear liquid	0.6	1 tube
Polymerase (TaqF)	colorless clear liquid	0.06	1 tube
Positive Control DNA *Mycoplasma pneumoniae / Chlamydophila pneumoniae / Prothrombin* (C+$_{M.p. / C.p / P}$)	colorless clear liquid	0.1	2 tubes
TE-buffer	colorless clear liquid	0.5	2 tubes
Negative Control (C–)*	colorless clear liquid	1.2	1 tube

* must be used in the extraction procedure as Negative Control of Extraction (see **RIBO-sorb**, REF K2-1-Et-100-CE or **RIBO-prep** REF K2-9-Et-100-CE protocols).

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

4. ADDITIONAL REQUIREMENTS

- Transport Medium for Storage and Transportation of Respiratory Swabs
- Flocked swabs with plastic shafts for nasopharyngeal swabs from inferior nasal meatus for children and adults.
- Rayon swabs with plastic shafts for oropharyngeal swabs for children and adults.
- Reagent for pretreatment of viscous fluids (sputum).
- Sterile saline or phosphate buffer for preconditioning autopsy material.
- Porcelain mortar and pestle for homogenize the autopsy material.
- Microcentrifuge (12,000 g).
- DNA extraction kit or the DNA extraction automatic station.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with filters (up to100 and 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany) iCycler iQ or iCycler iQ5 (Bio-Rad, USA); CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml) when working with PCR kit variant FRT-100 F:
  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 the temperature 2 to 8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid 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.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

NOTE: Obtaining samples of biological materials for PCR-analysis, transportation and storage are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*-FRT PCR kit is intended for analysis of the DNA extracted using DNA extraction kits from nasopharyngeal and oropharyngeal swabs, sputum (or tracheal aspirate), bronchial washing fluid or bronchoalveolar lavage, autopsy material, and whole blood.

Sampling

6.1. *Nasopharyngeal swabs*. Use sterile dry flocked swabs with plastic shafts for nasopharyngeal swabs. If the nasal cavity is full of mucus it is recommended to blow the nose before the procedure. Gently insert the swab along the external nasal wall to a depth of 2–3 cm towards the inferior nasal concha. Then move the swab slightly lower, insert it in the inferior nasal meatus under the inferior nasal concha, rotate, and remove along the external nasal wall. When the material is obtained, insert the swab into a sterile disposable tube with 500 µl of **Transport Medium for Storage and Transportation of Respiratory Swabs**, REF 957-CE. Break off the end of shaft to allow tight closing of the tube cup. Close the tube with the solution and the swab.

6.2. *Oropharyngeal swabs.* Use sterile dry rayon swabs with plastic shafts for oropharyngeal swabs. Rotate the swab over the surface of tonsils, palatine arches, and the posterior wall of the pharynx. When material is obtained, insert the swab in a sterile disposable tube with 500 µl of **Transport Medium for Storage and Transportation of Respiratory Swabs**, REF 957-CE. Break off the end of shaft to allow tight closing of the tube cup. Close the tube with the solution and the swab.

NOTE: It is recommended to combine the nasopharyngeal and oropharyngeal swabs in a single tube. For this purpose, place the ends of both shafts after sampling should be placed in a tube with 0.5 ml of **Transport Medium for Storage and Transportation of Respiratory Swabs,** REF 957-CE and studied as one sample. Store the samples at 2–8 °C for 3 days or at minus 24 to minus 16 °C for 1 week.

6.3. *Tracheal sputum or aspirate*. Sputum is taken in sterile hermitic disposable plastic containers after gargling the oral cavity with water. Trachea aspirates are get by traditional method and placed in sterile hermitic disposable plastic containers.

6.4. The samples of *bronchoalveolar lavage or bronchial washing fluid* are taken in disposable tightly closing polypropylene 5 ml volume cups (in order to avoid cells adhesion on the internal cup surface). Store the samples at 2–8 °C for 1 day or at minus 24 to minus 16 °C for 1 week.

6.5. *Autopsy material* is placed immediately in sterile disposable containers and either frozen after taking or studied within an hour. Store the samples at minus 68 °C for 1 year. Only one material freezing–thawing cycle is allowed.

6.6. *Whole blood* is taken in the Vacuette® tubes with EDTA solution or evaporation. Closed tube with material is turned several times to mix preservative. Use whole blood, collected on an empty stomach in the morning for analysis. Storage for 3 days at 2–8 °C is allowed.

NOTE: Whole blood is not to be used for acute respiratory infection diagnostics. **RIBO-sorb** nucleic acid extraction kit, REF K2-1-Et-50-CE can be use only for DNA extraction.

Pretreatment

6.7 *Nasal and oropharyngeal swabs.* Vortex the tube, then centrifuge it at 5,000 rpm for 5 s to sediment drops from the interior wall of the tube cap. Use 100 µl of sample for DNA extraction.

6.8 *Sputum or tracheal aspirate*. Use reagent **Mucolysin** REF 180-CE for sputum and aspirate pretreatment (for viscosity reduction). See the instruction manual to **Mucolysin** for a proper use. The pretreated sputum (100 µl) is used for DNA extraction. If it is necessary to repeat the test, the residue of sputum can be frozen.

6.9 *Bronchoalveolar lavage or bronchial washing fluid.* Mix the sample by inverting in the initial cup. Transfer 1 ml of the sample using filter tip to the 1.5 ml tube for the centrifugation at 10,000 rpm for 10 min. The supernatant is removed carefully by filter tip, reserving 200 µl over sediment. Sediment is to be resuspended in the residue of supernatant. Use 100 µl of obtained suspension for DNA extraction. If it is necessary to repeat the test, the residual material can be frozen.

6.10 *Autopsy material* is homogenized using sterile porcelain mortars and pestles. Then, prepare a 10 % suspension in a sterile saline or phosphate buffer. Transfer the suspension to a 1.5-ml tube and centrifuge at 10,000 rpm for 5 min. The supernatant (100 µl) is used for DNA extraction. If it is necessary to repeat the test, the residual suspension can be frozen.

7. WORKING CONDITIONS

AmpliSens® *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits:

- RIBO-sorb, REF K2-1-Et-100-CE
- RIBO-prep, REF K2-9-Et-100-CE
- NucliSENS easyMAG automated system (for details see Guidelines [2]).

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

8.2. Preparing the PCR

NOTE: The Positive Control of Amplification (C+) and Negative Control Amplification (NCA) (for control reagents purity and operator accurateness) are used at the amplification stage of each run. In addition Negative Control of Extraction (C-) is analyzed at the amplification stage.

8.2.1 Preparing tubes for PCR

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

1. Thaw the required number of tubes with **PCR-mix-1-FRT (F) *Mycoplasma pneumonia* / *Chlamydophila pneumoniae,* PCR-mix-2-FRT,** and **polymerase (TaqF)**, mix the reagents and then centrifuge briefly (1-2 sec) to sediment the drops.
   Take the required number of tubes/strips for amplification of the DNA obtained from clinical and control samples.
2. For N reactions, add to a new tube:
   10*(N+1) µl of PCR-mix-1-FRT (F) *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*,
   5.0*(N+1) µl of PCR-mix-2-FRT
   0.5*(N+1) µl of polymerase (TaqF).
   Vortex the tube, then centrifuge it briefly to sediment the drops. Transfer **15 µl** of the prepared mixture to each tube.
3. Using tips with aerosol filter, add **10 µl** of **DNA samples** obtained at the DNA extraction stage.
4. Carry out the control amplification reactions:
   NCA – Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification).
   C+ – Add **10 µl** of **Positive Control DNA *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae* / *Prothrombin* (C+$_{M.p. / C.p / P}$)** to the tube labeled C+ (Positive Control of Amplification).
   C– – Add **10 µl** of the sample extracted from **Negative Control (C–)**.

8.3 Amplification

1. Create a temperature profile on your instrument as follows (see Table 2):

Table 2

Amplification program for *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* DNA

	Rotor-type Instruments[1]			Plate-type Instruments[2]		
Step	**Temperature, °C**	**Time**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
1	**95**	**15 min**	**1**	**95**	**15 min**	**1**
2	**95**	**10 s**	**10**	**95**	**10 s**	**10**
	60	**20 s**		**60**	**25 s**	
	72	**10 s**		**72**	**25 s**	
3	**95**	**10 s**	**35**	**95**	**10 s**	**35**
	60	**20 s Fluorescence acquiring**		**60**	**25 s Fluorescence acquiring**	
	72	**10 s**		**72**	**25 s**	

Fluorescent signal is detected in the channels for the FAM, JOE, and ROX fluorophores. Amplification program for a specific model of instrument is specified in Guidelines [2].

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines [2].
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument. 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 (see Table 3).

Table 3

Target compliance with detection channels

Detection in the channel for the fluorophore		
FAM	**JOE**	**ROX**
Mycoplasma pneumoniae	Human DNA	*Chlamydophila pneumoniae*

Principle of interpretation is the following:

– *Mycoplasma pneumonia* and *Chlamydophila pneumoniae* DNA is **detected** if the *Ct* value determined in the results grid in the respective channel for the fluorophore (see table 2) are less than the boundary *Ct* value specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.

– *Mycoplasma pneumonia* and *Chlamydophila pneumoniae* DNA **is not detected** in a sample if the *Ct* value is not determined (fluorescence curve does not cross the threshold line) or exceed the boundary *Ct* value specified in the *Important Product Information Bulletin* in the respective channel for the fluorophore (see table 2), whereas the *Ct* value determined in the channel for the JOE fluorophore (IC – Human DNA) is less than the specified boundary *Ct* value.

– The result is **invalid** if the *Ct* value is not determined (absent) or greater than specified boundary *Ct* value in the channel for the FAM or ROX fluorophores, whereas the *Ct* value in the channel for the JOE fluorophore (IC) is absent or greater than the specified boundary *Ct* value. In such cases, the PCR analysis should be repeated starting from the DNA extraction stage. If the same result is obtained in the second run, re-sampling of material is recommended.

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

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

Table 4

Results for controls

Control	**Stage for control**	***Ct* value in the channel for the fluorophore**		
		FAM (*Mycoplasma pneumoniae*)	**JOE** (Human DNA)	**ROX** (*Chlamydophila pneumoniae*)
C–	DNA extraction	Absent	Absent	Absent
NCA	PCR	Absent	Absent	Absent
C+	PCR	**<**boundary value	**<**boundary value	**<**boundary value

[1] For example, Rotor-Gene 3000/Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany).
[2] For example, iCycler iQ, iQ5 (Bio-Rad, USA), CFX96 (Bio-Rad, USA).

10. TROUBLESHOOTING

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

1. If the *Ct* value determined for the Positive Control of Amplification (C+) in the channels for detection of some target gene is greater than the boundary *Ct* value or absent, the amplification and detection should be repeated for the clinical samples that were negative in this channel and for Positive Control of Amplification (C+) samples.
2. If the *Ct* value determined for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C–) in the channels for detection of some target gene is less than the boundary *Ct* value, the analysis should be repeated from the extraction stage for all samples in which the given target was detected in order to exclude the consequence of possible contamination

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*-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® *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*-FRT** PCR kit are to be stored at 2–8 °C (except for PCR-mix-1-FRT (F) *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*, PCR-mix-2-FRT and polymerase (TaqF)) when not in use. All components of the **AmpliSens® *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*-FRT** PCR kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-1-FRT (F) *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*, PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C

NOTE: PCR-mix-1-FRT (F) *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae* is to be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

Biological material	Pathogen agent	Material volume, µl	Nucleic acid extraction kit	Sensitivity, GE/ml[3]
Nasopharyngeal and oropharyngeal mucosa and sputum treated with mucolisin	*Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*	100	**RIBO-sorb**	$1x10^3$
			RIBO-prep	$5x10^2$
			NucliSENS easyMAG	$5x10^2$

13.2. Specificity

The analytical specificity of **AmpliSens® *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*-FRT** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The clinical specificity of **AmpliSens® *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*-FRT** PCR kit makes it possible to detect DNA of the specific fragments of the claimed pathogens. The specificity of this kit was confirmed by investigation of the following reference strains: *Streptococcus* spp., *Moraxella catarrhalis*, *Staphilococcus aureus*, *Staphilococcus saprophiticus*, *Haemophilus influenzae*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Mycobacteria tuberculosis* 27294 105, *Neisseria flava*, *Neisseria sicca*, *Neisseria mucosa*, *E. coli* ATCC, NCTC, *Enterococcus faecalis*, *Legionella pneumophila*, *Shigella flexneri*, *Shigella sonnei*, *Salmonella enteritidis*, *Yersinia enterocollitica*, *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica* as well as human genomic DNA. Activity of the components of PCR kit is absence in point of strains *Chlamydophila arginini*, *Chlamydophila pecorum*, *Chlamydia trachomatis*, *Chlamydia muridarum*, *Chlamydia suis*, *Chlamydophila abortus*, *Chlamydophila psittaci*, *Mycoplasma arginini*, *Mycoplasma mycoides* (*subspecies capri*), *Mycoplasma hyorinis, Mycoplasma bovigenitalium*, *Mycoplasma bovine*, *Mycoplasma salivarium*, *Mycoplasma faucium*, *Mycoplasma gallisepticum*, *Mycoplasma sinoviae*, *Mycoplasma genitalium*, *Mycoplasma hominis*.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2010.
2. Guidelines to the **AmpliSens® *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*-FRT** PCR kit for qualitative detection of *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* DNA in the biological material by 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 **AmpliSens® *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
26.02.15 ME	Text	Corrections according to the template. Reference numbers for RIBO-sorb and RIBO-prep were changed to REF K2-1-Et-100-CE and REF K2-9-Et-100-CE
	1. Intended use	The type of biological material – tracheal aspirate – was added
	2. Principle of PCR detection	The text was corrected in part of using endogenous internal control
	6. Sampling and handling	The type of biological material – tracheal aspirate – was added. Grammar corrections
	8. Protocol	Rotor-Gene Q instrument was added in footnote 1, CFX96 instrument was added in footnote 2
	13. Specifications	In the table with sensitivity the clinical material was changed to biological material, the unit of measurement of material volume was corrected from ml to µl
	14. References	The reference to guidelines was corrected
27.06.17 ME	4. Additional requirements	Text corrections
	6. Sampling and handling	
05.12.18 EM	2. Principle of PCR detection	The table with targets and the information about the enzyme UDG were added
	Through the text	The text formatting was changed
27.02.20 PM	Footer	The phrase “Not for use in the Russian Federation” was added
01.03.21 EM	—	The name, address and contact information for Authorized representative in the European Community was changed

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[3] Genome equivalents (GE) of the pathogen agent per 1 ml of a sample.