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IVD

For Professional Use Only

AmpliSens® *Cov*-Bat-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *Cov*-Bat-FRT PCR kit is an *in vitro* nucleic acid amplification test for detection of RNA of *coronaviruses* causing severe respiratory infections MERS-*Cov* (Middle East respiratory syndrome *coronavirus*) and SARS-*Cov* (Severe acute respiratory syndrome *coronavirus*) in the biological material (nasopharyngeal and oropharyngeal swabs, blood plasma, sputum, faeces) by using real-time hybridization-fluorescence detection of amplified products.

Reagents Kit AmpliSens® *Cov*-Bat-FRT can be used without distinction of form and presence of manifestation.

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

2. PRINCIPLE OF PCR DETECTION

MERS-*Cov* and SARS-*Cov coronaviruses* RNA detection by the polymerase chain reaction (PCR) is based on the amplification of the regions of upE area of MERS-*Cov coronavirus* genome and Pol gene of SARS-*Cov coronavirus* 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® *Cov*-Bat-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-87-rec). 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. The positive control of extraction and reverse transcription of RNA (PCE) is the tube in which the Positive Control MERS-*Cov* / SARS-*Cov* (non-infectious recombinant preparation) is added. The negative control of RNA extraction (C–) is the tube in which the Negative Control (C–) is added. To obtain complementary DNA (cDNA) on RNA matrix, reverse transcription reaction is required.

AmpliSens® *Cov*-Bat-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® *Cov*-Bat-FRT PCR kit is produced in 1 form:

AmpliSens® *Cov*-Bat-FRT PCR kit variant FRT-50 F, **REF** R-V65-F-CE.

AmpliSens® *Cov*-Bat-FRT PCR kit variant FRT-50 F includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-FL MERS-*Cov* / SARS-*Cov*	colorless clear liquid	0.6	1 tube
PCR-buffer-B	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
Positive Control MERS-*Cov* / SARS-*Cov* / STI ($C+_{MERS\text{-}Cov\ /\ SARS\text{-}Cov\ /\ STI}$)	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	2 tube
Internal Control STI-87-rec (IC)**	colorless clear liquid	0.12	5 tubes
Positive Control MERS-*Cov* / SARS-*Cov****	colorless clear liquid	0.1	1 tube

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

** add 10 µl of Internal Control during the RNA extraction procedure directly to the sample/lysis mixture (see RIBO-prep, **REF** K2-9-Et-50-CE protocol).

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

AmpliSens® *Cov*-Bat-FRT PCR kit variant FRT-50 F is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- Transport medium for storage and transportation of respiratory swabs.
- Reagent for pretreatment of viscous fluids (sputum, aspirates).
- Flocked or fiber swabs for collecting specimens from kids and adults.
- A disposable needle (0.8–1.1 mm in diameter) and vacuum system for blood plasma production.
- 0.9 % saline solution or 0.01 M potassium-phosphate buffer (pH 7.0) for pretreatment of autopsy material or in case of viral cultures testing.
- RNA extraction kit.
- Reverse transcription kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).

- Sterile RNase-free pipette tips with aerosol barriers (up to 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 iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA), CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-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 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 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 protective gloves and laboratory cloths, and protect eye 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 compliance with appropriate biosafety practices.
- Clean and disinfect all specimens or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid specimens and reagents contact with the skin, eyes, and mucous membranes. If

these solutions come into contact, rinse 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 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

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® ***Cov*-Bat-FRT** PCR kit is intended for analysis of RNA extracted with RNA extraction kits from the biological material.

For the study of the patient is advised to take at least two types of biological material (material taken from the respiratory tract, blood plasma, and faeces in the presence of symptoms of gastrointestinal tract disease):

- nasopharyngeal and posterior wall of oropharynx swabs (in the presence of symptoms upper respiratory tract involvement),
- sputum (or tracheal aspirates) (in the presence of symptoms lower respiratory tract involvement),
- bronchoalveolar lavage or washdown waters of the bronchi (if the patient is intubated),
- blood plasma,
- faeces (in the presence of symptoms of gastrointestinal tract disease),
- autopsy material (fragments of the affected part of the lung, bronchi) in the case of death.

Nasopharyngeal and oropharyngeal Swabs recommended combining in a tube and exploring as one sample. In this case take the first swabs from the mucous membranes of the inferior nasal meatus, and then from the oropharynx (use new probe tools for each of samples). The working ends of the probe tools placed in a tube with 0.5 ml of transport medium for storage and transport of respiratory swabs and investigated as one sample.

Sampling

6.1 *Nasopharyngeal swabs* are taken from children and adults using a dry sterile nasopharyngeal probe tool with a flocked tip, and a plastic applicator (if the cavity is full of mucus it is recommended to blow the nose well before starting the procedure). The probe tool is gently inserted into the cavity along the outer nose cavity paries at a depth of 2–3 cm up to the lower concha of cranium. Then the tool is slightly moved downwards, inserted into the inferior nasal meatus under the lower concha of cranium, and after a rotational movement it is removed from the nose along the outer nose cavity paries. The depth of inserting the probe tool is about 3–4 cm for children, and 5–6 cm for adults. After sampling the material the end of the probe tool with the swab is put into a sterile disposable tube containing 500 µl of Transport Medium for Storage and Transportation of Respiratory Swabs (**REF** 957-CE) up to the point of breaking the tool. The flexible part of the tool is coiled. Then the handle of the tool is to be gently broken off, during this procedure the tube is to be covered up with its cap. Then the tube is closed tightly and labeled. It is allowable to use dry sterile polystyrol probe tools with a viscose tip for taking the material from adults.

6.2 *Oropharyngeal swabs* are taken with dry sterile probe tools with viscose tips with rotational movements from the surface of the tonsils, arches of the palate, and from the back paries of the oropharynx. After taking the material, the working part of the probe tip with a viscose tip is put into a sterile disposable tube containing 0.5 ml of transport medium and the probe with the nasopharyngeal swab. The end of the probe is to be broken off so that the tube could be closed tightly. Then the tube with the solution and the working part of the probe tool is closed and labeled. Store the samples before study at 2–8 °C for 3 days or at not more than minus 16 °C for a long time.

6.3 *Sputum* is taken into pressureproof disposable plastic containers after the mouth cavity was gargled with water. *Tracheal aspirates* are taken using traditional methods and put into sterile pressureproof disposable plastic containers. Store the samples before study at 2–8 °C for 1 day or at not more than minus 16 °C for a long time.

6.4 *Bronchoalveolar lavage* and *washdown waters of the bronchi* are taken into disposable tightly screwed down polypropylene reservoirs with the volume not less than 5 ml. Store the samples before study at 2–8 °C for 1 day or at not more than minus 16 °C for a long time.

6.5 The *autopsy material* is put into sterile disposable containers and examined within 1 hour, or is frozen right after taking. The material may be frozen and thawed only once. Store the samples before study at not more than minus 16 °C for 7 day or at not more than minus 68 °C for 1 year. Only one material freezing – defrosting is allowed.

6.6 For *blood plasma* production blood is taken under fasting conditions from ulnar veins with a disposable needle (0.8–1.1 mm in diameter) into a vacuum system like Venoject (with EDTA), or Vacuette (lilac caps – 6 % EDTA). The tube is to be rotated gently several times (for mixing with the anticoagulant). Blood plasma is produced through centrifuging the tubes with whole blood at 3,000 rpm using a microcentrifuge during 20 min at room temperature. Then not less than 1 ml of blood plasma is taken with separate tips with aerosol filters into sterile 1.5 ml-tubes. 100 µl of plasma sample is used for the RNA extraction. Store the samples at 2–8 °C for 3 days, at not more than minus 16 °C for 1 month, at not more than minus 68 °C for 1 year.The material may be frozen and thawed only once.

6.7 *Faeces* samples are taken into a sterile pot or bed-pan. Then approximately 1 gram of faeces is put into a disposable pressureproof polypropylene reservoir. Store the samples before study at 2–8 °C for 1 day or at not more than minus 16 °C for a long time. The material may be frozen and thawed only once.

Pretreatment

6.8. *Nasopharyngeal and oropharyngeal swabs.* The content of the closed tube is to be mixed using a vortex and centrifuged during 5 s at 5,000 rpm using a microcentrifuge in order to delete drops from the inner surface of the tube's cap. 100 µl of the sample is taken for the RNA extraction.

6.9. *Sputum or tracheal aspirates*. In order to reduce the viscosity of sputum into a container with sputum add equal amount of sputum reagent Mucolysin (**REF** 957-CE). Then incubation at room temperature until enlightenment sputum (no more than 20 min). 100 µl of the prepared sputum is used for the RNA extraction, the remained sputum is frozen if it is necessary to carry out the analysis for the second time.

6.10. *Bronchoalveolar lavage or washdown waters of the bronchi*. The sample is mixed through rotating in the initial reservoir. 1 ml of the sample is taken using an automatic pipette with a filter tip and put into a 1.5 ml-tube for centrifuging at 10,000 rpm during 10 min. The supernatant fluid is taken gently using a filter tip. 200

µl of the fluid is to be left over the pellet, the pellet is to be resuspended in the fluid. 100 µl of the suspension is used for the RNA extraction. If it is necessary to repeat the analysis, the remained material is frozen.

6.11. *The autopsy material* is homogenated using porcelain mortars and pestles, then 10 % suspension is prepared using sterile saline or phosphate buffer. The suspension is put into a 1.5 ml-tube and centrifuged at 10,000 rpm during 5 min. 100 µl of supernatant fluid is used for the RNA extraction. If it is necessary to repeat the analysis, the remained suspension is frozen.

6.12. 4.0 ml of saline is to be added to the sample of *faeces* (the volume up to 1.0 ml (0.4-1.0 gram)) so that 10–20 % suspension appeared (aquose faeces may be used without making the suspension). The suspended faeces is shaken up intensively using a vortex until the suspension arrears. The obtained suspension is clarified in one of the two following ways:

- Centrifuging the faeces suspension at 3,000 rpm during 20 min. The supernatant (clarified extract of faeces) is used for the RNA extraction.
- Carrying out the process of rapid filtration of the faeces suspension. Two 1 ml-tips (one of them is to be with an aerosol filter, the other one – without any filter) and a polystyrol cotton tipped applicator are used for the rapid filtration. It is necessary to prepare the filtration station beforehand: the cotton tip is cut off the applicator, put into the tip without an aerosol filter, and pushed to the narrow part of the tip as far as it can go using another clean tip. 0.5 ml of the faeces suspension is taken using an automatic 1 ml-pipette with a filter tip. The tip with the suspension is put tightly up to the stop into the prepared tip with the cotton piece. The filtration of the suspension is carried out from the pipette tip with a filter throuth the tip with the cotton piece into a new mictocentrifuge 1.5 ml-tube under pressure of the pipette piston. If there are any difficulties during the filtration process it is recommended to reduce the concentration of the faeces suspension. The filtrate (0.5 ml) is used for the RNA filtration. The material may be frozen and thawed only once.

Store the samples at 2–8 °C for 1 week or at from minus 24 to minus 16 °C for a long time. The material may be frozen and thawed only once.

7. WORKING CONDITIONS

AmpliSens® *Cov*-Bat-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA extraction

To work with RNA is necessary to use only sterile disposable plastic consumables with special RNase-free, DNase-free markings.

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

– RIBO-prep, **REF** K2-9-Et-50-CE.

Extract the RNA according to the manufacturer's protocol.

RNA extraction is carried out from 100 µl prepared samples of biological material except faeces. For working with faeces for RNA extraction used prepared suspension consisting of 50 µl C– and 50 µl faeces.

The extraction process of each individual sample is carry out with the Internal Control (Internal Control STI-87-rec). The positive control of extraction and reverse transcription of RNA is the tube contains the 90 µl Negative Control (C–) and the 10 µl Positive Control MERS-*Cov* / SARS-*Cov*.

8.2. Reverse transcription

It is recommended to use the following kit for complementary DNA (cDNA) synthesis from RNA:

– REVERTA-L, **REF** K3-4-50-CE.

8.3. Preparing PCR

8.3.1 Preparing tubes for PCR

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

1. Thaw the required number of tubes with **PCR-mix-FL MERS-*Cov* / SARS-*Cov.*** Mix the content of the tubes with **PCR-mix-FL MERS-*Cov* / SARS-*Cov,* PCR-buffer-B,** and **Polymerase (TaqF)**, then centrifuge them briefly (1–2 s).
2. Take the required number of tubes or strips for the cDNA amplification of the test and control samples.
3. For N reactions mix in a separate tube (see Table 1):

   10*(N+1) µl of **PCR-mix-FL MERS-*Cov* / SARS-*Cov*;**

   5*(N+1) µl of **PCR-buffer-B;**

   0,5*(N+1) µl of **Polymerase (TaqF).**

Table 1

Scheme of reaction mixture preparation for variant FRT-50 F

	Reagent volume for specified number of reactions		
Reagent volume per one reaction, µl	**10.0**	**5.0**	**0.5**
Number of reactions[1]	**PCR-mix-FL MERS-*Cov* / SARS-*Cov***	**PCR-buffer-B**	**Polymerase(TaqF)**
6	**60**	**30**	**3.0**
8	**80**	**40**	**4.0**
10	**100**	**50**	**5.0**
12	**120**	**60**	**6.0**
14	**140**	**70**	**7.0**
16	**160**	**80**	**8.0**
18	**180**	**90**	**9.0**
20	**200**	**100**	**10.0**
22	**220**	**110**	**11.0**
24	**240**	**120**	**12.0**
26	**260**	**130**	**13.0**
28	**280**	**140**	**14.0**
30	**300**	**150**	**15.0**
32	**320**	**160**	**16.0**

4. Vortex the tube, then centrifuge briefly. Transfer **15 µl** of the prepared mixture to each tube.

5. Add **10 µl** of **cDNA samples** obtained in the RNA reverse transcription reaction into the prepared tubes.

6. Carry out the control amplification reactions:

NCA - Add **10 µl** of **C–** to the tube labeled NCA (Negative Control of Amplification)

C+$_{\text{MERS-}Cov\text{ / SARS-}Cov\text{ / STI}}$ - Add **10 µl** of **Positive Control MERS-*Cov* / SARS-*Cov* / STI (C+$_{\text{MERS-}Cov\text{ / SARS-}Cov\text{ / STI}}$)** to the tube labeled C+$_{\text{MERS-}Cov\text{ / SARS-}Cov\text{ / STI}}$

C– - Add **10 µl** of **the sample extracted from the Negative Control reagent** to the tube labeled C–

PCE - Add **10 µl** of **the sample extracted from the Positive Control MERS-*Cov* / SARS-*Cov* reagent** to the tube labled PCE

8.3.2. Amplification

1. Create a temperature profile on your instrument according of the following program:

[1] Number of test samples (N) including the 2 control of extraction stage + 2 controls of amplification + 1 extra reaction (N+2+2+1).

Table 2

Amplification program

	***Rotor-type instruments*[2]**			***Plate-type instruments*[3]**		
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
	54	20 s		54	25 s	
	72	10 s		72	25 s	
3	95	10 s	35	95	10 s	35
	54	20 s Fluorescence acquiring		54	25 s Fluorescence acquiring	
	72	10 s		72	25 s	

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

2. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin* and Guidelines [2].
3. Insert tubes into the reaction module of the device.

It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them into the instrument.

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 software of the used real-time PCR instrument by measuring fluorescence signal accumulation in three channels:

- The signal of the IC cDNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the SARS-*Cov* cDNA amplification product is detected in the channel for the JOE fluorophore.
- The signal of the MERS-*Cov* cDNA amplification product is detected in the channel for the ROX fluorophore.

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

Principle of interpretation is the following:

- **MERS-*Cov*** RNA is **detected** if the *Ct* value determined in the result grid in the channel for the ROX fluorophore is less than the boundary Ct value specified in the

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

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.

– **SARS-*Cov*** RNA is **detected** if the *Ct* value determined in the result grid in the channel for the ROX fluorophore is 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.
– **MERS-*Cov*** and **SARS-*Cov*** RNA is **not detected** in a sample if *Ct* value is not determined (absent) in the channels for ROX and JOE fluorophores, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary Ct value specified in the *Important Product Information Bulletin*.
– The result is **invalid** if *Ct* value is not determined (absent) in the channel for JOE or ROX fluorophores, whereas the *Ct* value in the channel for the FAM fluorophore is not determined (absent) or greater than the specified boundary Ct value. In such cases, PCR analysis should be repeated starting from the RNA extraction stage. If the same result is obtained in the second run, re-sampling of material is recommended.
– The result is **equivocal** if the *Ct* value determined in the channel for ROX or JOE fluorophore is greater than the boundary Ct value specified in the *Important Product Information Bulletin*, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary Ct value specified in the *Important Product Information Bulletin.* In such cases, PCR analysis should be repeated starting from the RNA extraction stage. If the same result is obtained, the sample is considered positive.

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

The result of the analysis is considered reliable only if the results obtained for Negative Controls of amplification as well as for the Negative Control of extraction, reverse transcription, and amplification of cDNA are correct (Table 3).

Table 3

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore		
		FAM	**JOE**	**ROX**
C–	Extraction and reverse transcription of RNA	**<**boundary value	Absent	Absent
PCE	Extraction and reverse transcription of RNA	**<**boundary value	**<**boundary value	**<**boundary value
NCA	PCR	Absent	Absent	Absent
C+	PCR	**<**boundary value	**<**boundary value	**<**boundary value

10. TROUBLESHOOTING

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

1. If the threshold cycle (*Ct*) value is absent or more the boundary value for the positive control of extraction (PCE) or the positive control of PCR (C+) for the channels for **JOE or ROX** fluorophores, it is necessary to repeat amplification for all samples in which the RNA of the virus was not detected in the respective channel.
2. If the *Ct* value is determined for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C–) in the channels for **JOE or ROX** fluorophores, the amplification should be repeated for all the samples in which the RNA of the virus was detected in the respective channel.

11. TRANSPORTATION

AmpliSens® *Cov*-Bat-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® *Cov*-Bat-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-FL MERS-*Cov* / SARS-*Cov*, PCR-buffer-B, and polymerase (TaqF)). All components of the **AmpliSens® *X*-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.

PCR-mix-FL MERS-*Cov* / SARS-*Cov,* PCR-buffer-B, and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C

PCR-mix-FL MERS-*Cov* / SARS-*Cov* is to be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

Biological material	Pathogen agent	Nucleic acid extraction kit	PCR kit	Sensitivity, GE/ml[4]
Nasopharyngeal and oropharyngeal swabs, blood plasma, sputum	MERS-*Cov*	RIBO-prep	PCR kit FRT-50 F	1 x 10^3
	SARS-*Cov*	RIBO-prep	PCR kit FRT-50 F	1 x 10^3
Faeces	MERS-*Cov*	RIBO-prep	PCR kit, FRT-50 F, rotor-type instruments	1x10^3
	SARS-*Cov*	RIBO-prep	PCR kit FRT-50 F, rotor-type instruments	1x10^3
Faeces	MERS-*Cov*	RIBO-prep	PCR kit FRT-50 F, plate-type instruments	1x10^4
	SARS-*Cov*	RIBO-prep	PCR kit FRT-50 F, plate-type instruments	1x10^4

13.2. Specificity

The analytical specificity of **AmpliSens® *Cov*-Bat-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 specific activity of the PCR kit is proved by the examination of the positive control sample of the MERS-*Cov coronavirus* RNA (*hCov*-EMC upE-Assay: IVT-RNA) which is recommended by the WHO for the screening analysis (Institute of Virology, University of Bonn Medical Centre, Germany), and SARS-*Cov coronavirus* RNA causing a severe acute respiratory syndrome – SARS (University of Frankfurt, Germany).

The activity of the kit components is absent in reference to the following *coronavirus* strains: *feline coronavirus – FC0* and *FC1*, *canine coronavirus – CCV, coronavirus* causing *avian infectious bronchitis*. Nonspecific reactions with *coronaviruses*, isolates which are main causative pathogens of human ARD (*Cov*-E229, *Cov*-OC43, *Cov*-HKUI, *Cov*-NL63), and also with cDNA/DNA of strains and isolates of main causative pathogens of human respiratory infections (*influenza virus* A and B, respiratory syncytial virus, *methapneumovirus*, *parainfluenza viruses*, *rhinoviruses*, *bocavirus*, *adenoviruses, enteroviruses*, *Streptococcus* spp., *Staphylococcus aureus*, *Mycoplasma pneumoniae*,

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

Chlamydophila pneumoniae, *Haemophilus influenzae, Moraxella catarrhalis, Legionella pneumophila*), of the normal microflora of the nasal and oropharyngeal cavities and human cDNA/DNA were not registered.

The clinical specificity of **AmpliSens® *Cov*-Bat-FRT** PCR kit was confirmed in laboratory clinical trials.

13.3. Diagnostic Characteristics

The results of performance testing reagent kit:

Samples Description	Biological material	Number of samples	Effectiveness of using AmpliSens® *Cov*-Bat-FRT reagents kit
Biological material containing[5)] RNA of MERS-*Cov coronavirus*	Nasopharyngeal and oropharyngeal swabs	100 pcs	100 % Positive
	Sputum	100 pcs	100 % Positive
	Blood plasma	100 pcs	100 % Positive
	Faeces	100 pcs	100 % Positive
Biological material containing [5)] RNA of SARS-*Cov coronavirus*	Nasopharyngeal and oropharyngeal swabs	100 pcs	100 % Positive
	Sputum	100 pcs	100 % Positive
	Blood plasma	100 pcs	100 % Positive
	Faeces	100 pcs	100 % Positive
Biological material does not contain[6)] RNA of MERS-*Cov,* SARS-*Cov coronavirus*	Nasopharyngeal and oropharyngeal swabs	100 pcs	100 % Negative

In accordance with the submitted data the **diagnostic sensitivity** of the reagents kit for all types of biological material is 98-100 % with a confidence coefficient of 90 %.

The **diagnostic specificity** of the reagents kit is 98-100 % with a confidence coefficient of 90 %.

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.

[5)] The samples containing MERS-*Cov* and SARS-*Cov* coronaviruses is model samples of biological material containing the recombinant quality control sample.

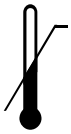
[6)] The samples do not contain MERS-Cov and SARS-Cov coronaviruses is samples of biological material from the patients with respiratory infections, containing influenza virus A/H1N1pdm2009, parainfluenza viruses, rhinoviruses, and that has been proven in testing with reagents kits AmpliSens® Influenza virus A/B-FRT, AmpliSens® Influenza virus A/H1-swine-FRT and AmpliSens® ARVI-screen-FRT

2. Guidelines to the **AmpliSens® *Cov*-Bat-FRT** PCR kit for detection of RNA of *coronaviruses* causing severe respiratory infections MERS-*Cov* (Middle East respiratory syndrome *coronavirus*) and SARS-*Cov* (Severe acute respiratory syndrome *coronavirus*) in the biological material (nasopharyngeal and oropharyngeal swabs, blood plasma, sputum, faeces) 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 **AmpliSens® *Cov*-Bat-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	**PCE**	Positive control of extraction
	Upper limit of temperature	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	Positive control of amplification
	Keep away from sunlight	**IC**	Internal control