# AmpliSens® *Cov*-Bat-FRT PCR kit

IVD

For Professional Use Only

# Instruction Manual

## KEY TO SYMBOLS USED

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

## 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*-like viruses (Severe acute respiratory syndrome *coronavirus*, Severe acute respiratory syndrome *coronavirus*-2) in the biological material (nasopharyngeal and oropharyngeal swabs, sputum or tracheal aspirates, bronchoalveolar lavage or bronchial washing fluids, blood plasma, feces, autopsy material) and in the environmental samples (water sample concentrates, washes from environmental objects) in order to prevent coronavirus infection in humans by using real-time hybridization-fluorescence detection of amplified products.

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

**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 RNA extraction from the samples of test material with the exogenous internal control sample (Internal Control STI-87-rec (IC)), RNA reverse transcription and simultaneous amplification of cDNA fragments of the detected *coronaviruses* (MERS-*Cov* and SARS-*Cov*-like viruses (SARS-*Cov*, SARS-*Cov*-2)) and cDNA of the internal control with hybridization-fluorescence detection. Exogenous internal control (Internal Control STI-87-rec (IC)) allows to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition.

RNA reverse transcription and amplification of cDNA fragments with the use of specific primers and Taq-polymerase enzyme are performed with the RNA samples obtained at the extraction stage. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

**AmpliSens® *Cov*-Bat-FRT** PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed 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 (dUTP).

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

Table 1

| Channel for fluorophore | FAM | JOE | ROX |
|---|---|---|---|
| DNA-target | IC cDNA | SARS-*Cov*-like viruses (SARS-*Cov*, SARS-*Cov*-2) cDNA | MERS-*Cov* cDNA |
| Target gene | Artificially synthesized sequence | RdRp gene | upE region |

## 3. CONTENT

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

**variant FRT-50 F**, REF H-2242-1-CE.

**Variant FRT-50 F** includes:

| Reagent | Description | Volume, ml | Quantity |
|---|---|---|---|
| **PCR-mix-FL MERS-*Cov* / SARS-*Cov*** | clear liquid from colorless to blue grey colour | 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+$_{\text{MERS-Cov / SARS-Cov / STI}}$)** | 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 tubes |
| **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.

**Variant FRT-50 F** is intended for 55 reactions (including controls).

## 4. ADDITIONAL REQUIREMENTS

*For sampling and pretreatment*

- Transport medium for storage and transportation of respiratory swabs.
- Reagent for pretreatment of viscous fluids (sputum, aspirates).
- 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).
- Flocked or fiber swabs for collecting nasopharyngeal 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 and feces.

*For RNA extraction, reverse transcription and amplification*

- RNA extraction kit.
- Reverse transcription kit.
- Sterile RNase-free pipette tips with filters (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 iQ5 (Bio-Rad, USA); CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene 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;
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

## 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 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 samples 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 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

**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® *Cov*-Bat-FRT** PCR kit is intended for analysis of RNA extracted with RNA extraction kits from the biological material (nasopharyngeal and oropharyngeal swabs, sputum or tracheal aspirates, bronchoalveolar lavage or bronchial washing fluids, blood plasma, feces, autopsy material) and environmental samples (water sample concentrates, washes from environmental objects).

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

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

**NOTE:** It is recommended to combine nasopharyngeal and oropharyngeal swabs in a single tube. For this purpose, first take the swabs from the mucous membrane of inferior nasal meatus and oropharynx using different swabs and then place the ends of both shafts into one tube containing 500 µl of **Transport Medium for Storage and Transportation of Respiratory Swabs** (REF 959-CE, REF 957-CE, REF 958-CE) and analyze them as a single sample.

***Sampling***

*Nasopharyngeal swabs* are collected with sterile dry flocked swab with plastic shaft. 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. The total depth of insertion of the swab should be approximately half of the distance from the nostril to the ear hole (3-4 cm for children and 5-6 cm for adults).

When the material is obtained, insert the working part of the swab into a sterile disposable tube with 500 µl of **Transport Medium for Storage and Transportation of Respiratory Swabs** (REF 959-CE, REF 957-CE, REF 958-CE), the flexible part of the swab is minimized by a spiral, then, covering the top of the tube with a lid, the handle of the swab is lowered down, achieving complete breaking off the end of shaft. Close and mark the tube with the solution and the swab.

It is allowable to use dry sterile polystyrol swabs with a viscose tip for collecting material from adults.

*Oropharyngeal swabs* are collected using sterile dry rayon swabs by rotating the swab over the surface of tonsils, palatine arches, and posterior wall of pharynx.

When the material is obtained, insert the working part of the swab into a sterile disposable tube with 500 µl of **Transport Medium for Storage and Transportation of Respiratory Swabs** (REF 959-CE, REF 957-CE, REF 958-CE). Break off the end of shaft to allow tight closing of tube cap. Close and mark the tube with the solution and the swab.

The test material can be stored before the analysis:

- at the temperature from 2 to 8 °C – for 3 days;
- at the temperature from minus 24 to minus 16 °C – for a long time.

*Sputum or tracheal aspirate* is collected into sterile hermetic disposable plastic containers after gargling the oral cavity with water.

The test material can be stored before the analysis:

- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for a long time.

*Bronchoalveolar lavage or bronchial washing fluid* is collected into disposable tightly screwed polypropylene containers with a volume no less than 5 ml.

The test material can be stored before the analysis:

- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for a long time.

*Autopsy material* is placed into sterile disposable containers and investigated within 1 hour or frozen immediately after collection.

The test material can be stored before the analysis:

- at the temperature from minus 24 to minus 16 °C – for 1 week;
- at the temperature ≤ – 68 °C – for a long time.

Only one freeze-thawing cycle is acceptable.

*Blood plasma.* Blood should be taken after overnight fasting 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 obtained through centrifuging the tubes with whole blood at 3,000 rpm using a microcentrifuge during 20 min at room temperature. Then no 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.

The test material can be stored before the analysis:

- at the temperature from 2 to 8 °C – for 3 days;
- at the temperature from minus 24 to minus 16 °C – for 1 month;
- at the temperature ≤ – 68 °C – for 1 year.

Only one freeze-thawing cycle is acceptable.

*Feces* samples are taken into a sterile pot or bed-pan. Then approximately 1 gram of feces is placed into disposable tightly screwed polypropylene container.

The test material can be stored before the analysis:

- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for a long time.

Only one freeze-thawing cycle is acceptable.

*Water sample concentrates* are collected according to state and local authorities' requirements.

Water sample concentrates can be stored before the PCR analysis:

- at the temperature from 2 to 8 °C – for 3 days;
- at the temperature from minus 24 to minus 16 °C – for 1 year.

Only one freeze-thawing cycle is required.

*Washes from environmental objects* are collected with a swab moistened in a sterile saline solution. The washing area of the flat surface is 5-10 $cm^2$. The working part of the swab should be placed in 0.5-ml tube with sterile saline solution. Break off and remove the upper end of shaft. 100 µl of the sample is used for RNA extraction.

Washes from environmental objects can be stored before the PCR analysis:

- at the temperature from 2 to 8 °C – for 3 days;
- at the temperature from minus 24 to minus 16 °C – for 1 year.

Only one freeze-thawing cycle is required.

The test material can be transported at 2–8 °C for 3 days.

***Pretreatment***

Pretreatment of *water sample concentrates and washes from environmental objects* is not required.

*Nasopharyngeal 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 lid. 100 µl of sample is taken for RNA extraction.

*Sputum or tracheal aspirates.* Use **Mucolysin** (REF 180-CE) reagent for viscous sputum pretreatment. In order to reduce the viscosity of sputum an equal amount of Mucolysin reagent should be added into container with sputum 100 µl of sputum is used for the RNA extraction after incubation at room temperature until the sputum becomes clear (no more than 20 min). The residual sputum should be frozen if it is necessary to repeat the analysis.

*Bronchoalveolar lavage or bronchial washing fluid.* The sample should be mixed by inverting in the initial vessel. Using a tip with aerosol filter, transfer 1.0 ml of sample to a 1.5-ml tube and centrifuge it for 10 min at 10,000 rpm. Decant the supernatant leaving 200 µl of liquid above the pellet. Resuspend the pellet in 200 µl of supernatant and take 100 µl of the suspension for RNA extraction. The residual material should be frozen if it is necessary to repeat the analysis.

*Autopsy material.* The sample is homogenized using sterile porcelain mortars and pestles. Then, prepare a 10 % suspension in 0.9 % sodium chloride solution (sterile saline solution) or phosphate buffer solution (PBS). 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 RNA extraction. The residual suspension should be frozen if it is necessary to repeat the analysis.

*Feces.* 4.0 ml of saline is to be added to the sample of feces (the volume up to 1.0 ml (0.4-1.0 g)) until the obtaining of 10–20 % suspension (liquid feces are used without previous obtaining of suspension). The suspended feces should be mixed on vortex until the suspension is obtained.

The obtained suspension is clarified in one of the two following ways:

- Centrifuging the fecal suspension at 3,000 rpm during 20 min. The supernatant (clarified fecal extract) is used for the RNA extraction.
- Carrying out the process of express filtration of the fecal 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 express 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 fecal 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 through the tip with the cotton piece into a new microcentrifuge 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 fecal suspension. The filtrate (0.05 ml) is used for the RNA extraction.

The clarified fecal extract can be stored before the analysis:

- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for 1 week.

Only one freeze-thawing cycle is acceptable.

## 7. WORKING CONDITIONS

**AmpliSens® *Cov*-Bat-FRT** PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

## 8. PROTOCOL

### 8.1. RNA extraction

**NOTE:** Only sterile disposable plastic consumables with special RNase-free, DNase-free markings should be used for work with RNA.

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

- **RIBO-prep**, REF K2-9-Et-50-CE.

The RNA extraction of each test sample is carried out in the presence of **Internal Control STI-87-rec (IC).**

In the extraction procedure it is necessary to carry out the control reactions as follows:

**C–** Add **100 µl of Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction)

**PCE** Add **90 µl of Negative Control (C–)** and **10 µl** of **Positive Control MERS-*Cov* / SARS-*Cov*** to the tube labeled PCE (Positive Control of Extraction)

**NOTE:** Extract the RNA according to the manufacturer's protocol.

**NOTE:** RNA extraction is performed from 100 µl of prepared environmental samples and biological material except feces. When working with feces the prepared suspension consisting of 50 µl of Negative Control (C–) and 50 µl of feces is used for RNA extraction.

### 8.2. Reverse transcription

It is recommended to use the following kit for complementary DNA (cDNA) synthesis from the 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 the **cDNA** sample is **10 µl**.

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, cDNA and control samples into tubes.

1. Thaw the required number of tubes with **PCR-mix-FL MERS-*Cov* / SARS-*Cov***. Vortex the tubes with **PCR-mix-FL MERS-*Cov* / SARS-*Cov***, **PCR-buffer-B** and **polymerase (TaqF)** and then centrifuge them briefly (1–2 s).
2. Take the required number of tubes/strips for amplification of the cDNA obtained from test and control samples.
3. For N reactions, add to a new tube:
   **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 2

**Scheme of reaction mixture preparation**

| | 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 by RNA reverse transcription.
6. Carry out the control reactions:

**NCA** — Add **10 µl** of **TE-buffer** 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+**$_{\text{MERS-}Cov\text{ / SARS-}Cov\text{ / STI}}$ (Positive Control of Amplification).

**C–** — Add **10 µl** of **the sample extracted from the Negative Control (C–) reagent** to the tube labeled **C–** (Negative Control of Extraction).

**PCE** — Add **10 µl** of **the sample extracted from the Positive Control MERS-*Cov* / SARS-*Cov* reagent** to the tube labeled **PCE** (Positive Control of Extraction).

### 8.3.2. Amplification

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

Table 3

**Amplification program**

| | Rotor-type instruments[2] | | | | Plate-type instruments[3] | | | |
|---|---|---|---|---|---|---|---|---|
| **Step** | **Temperature, °C** | **Time** | **Fluorescent signal detection** | **Cycles** | **Temperature, °C** | **Time** | **Fluorescent signal detection** | **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** | **FAM, JOE, ROX** | | **54** | **25 s** | **FAM, JOE, ROX** | |
| | **72** | **10 s** | **—** | | **72** | **25 s** | **—** | |

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.

**NOTE:** 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 the software of the real-time PCR instrument used 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*-like viruses (SARS-*Cov*, SARS-*Cov*-2) 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 the specific level that corresponds to the presence (or absence) of a *Ct* value of the cDNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

– **MERS-*Cov*** RNA is **detected** if the *Ct* value determined in the results 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.
– **SARS-*Cov*-like viruses (SARS-*Cov*, SARS-*Cov*-2)** RNA is **detected** if the *Ct* value determined in the results grid in the channel for the JOE 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*-like viruses (SARS-*Cov*, SARS-*Cov*-2)** RNA is **not detected** in a sample if the *Ct* value is not determined (absent) in the channels for the 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 the *Ct* value is not determined (absent) in the channel for the 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, the 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 the 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, the PCR analysis should be repeated starting from the RNA extraction stage. If the same result is obtained, the sample is considered positive.

[1] Number of reactions including the number of test samples (N), the control of extraction stage and PCR, and one extra reaction (N+2+2+1).
[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).

**NOTE:** 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 the Positive and Negative Controls of amplification as well as for the Negative and Positive Controls of extraction and reverse transcription of RNA are correct (see Table 4).**

Table 4

**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 cases:

1. The *Ct* value determined for the Positive Control of Extraction (PCE) or the Positive Control of Amplification (C+) in the channels for the **FAM**, **JOE** or **ROX** fluorophores is absent or greater than the boundary *Ct* value. The amplification should be repeated for all the samples.
2. If the *Ct* value is determined for the Negative Control of Extraction (C–) in the channels for the **JOE** or **ROX** fluorophores and/or Negative Control of Amplification (NCA) in the channels for the **FAM**, **JOE** or **ROX** fluorophores. The amplification should be repeated for all samples in which specific RNA was detected.

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

## 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® *Cov*-Bat-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-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

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

## 13. SPECIFICATIONS

### 13.1. Analytical sensitivity

Table 5

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

### 13.2. Analytical 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 ARD (*influenza virus* A and B, *respiratory syncytial virus*, *methapneumovirus*, *parainfluenza viruses*, *rhinoviruses*, *bocavirus*, *adenoviruses, enteroviruses*, *Streptococcus* spp., *Staphylococcus aureus*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Haemophilus influenzae, Moraxella catarrhalis, Legionella pneumophila*), of the normal microflora of the nasopharyngeal 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.

[4] Sensitivity is expressed in genomic equivalents (GE) of the pathogen per 1 ml of sample.

### 13.3. Diagnostic characteristics

The results of reagent kit testing:

| Sample description | Test material | Number of samples | Effectiveness of using AmpliSens® *Cov*-Bat-FRT PCR 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 |
| | Feces | 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 |
| | Feces | 100 pcs | 100 % Positive |
| Biological material containing[5] RNA of SARS-*Cov*-2 *coronavirus* | Nasopharyngeal and oropharyngeal swabs | 51 pcs | 100 % Positive |
| | Sputum/tracheal aspirates | | |
| | Bronchoalveolar lavage | | |
| | Blood plasma | | |
| | Feces | | |
| | Autopsy material | | |
| Biological material not containing[6] RNA of MERS-*Cov,* SARS-*Cov coronavirus* | Nasopharyngeal and oropharyngeal swabs | 100 pcs | 100 % Negative |
| Biological material not containing[6] RNA of SARS-*Cov*-2 *coronavirus* | Nasopharyngeal and oropharyngeal swabs | 56 pcs | 100 % Negative |
| | Sputum/tracheal aspirates | | |
| | Bronchoalveolar lavage | | |
| | Blood plasma | | |
| | Feces | | |
| | Autopsy material | | |
| Environmental samples containing[7] SARS-*Cov*-2 RNA | Water sample concentrates | 50 pcs | 100 % Positive |
| | Washes from environmental objects | | |
| Environmental samples not containing[7] SARS-*Cov*-2 RNA | Water sample concentrates | 55 pcs | 100 % Negative |
| | Washes from environmental objects | | |

In accordance with the submitted data the **diagnostic sensitivity** of the reagent kit is 98-100 % with a confidence level of 90 % when detecting RNA of MERS-*Cov* and SARS-*Cov coronaviruses* for all types of biological material.
The **diagnostic sensitivity** of the reagent kit is 100 % (93-100 %) with a confidence level of 95 % when detecting RNA of SARS-*Cov*-2 *coronavirus*.
The **diagnostic specificity** of the reagent kit is 98-100 % with a confidence level of 90 % when detecting RNA of MERS-*Cov* and SARS-*Cov coronaviruses*.
The **diagnostic specificity** of the reagent kit is 100% (94-100 %) with a confidence level of 95 % when detecting RNA of SARS-*Cov*-2 *coronavirus*.

## 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.
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*-like viruses (Severe acute respiratory syndrome *coronavirus*, Severe acute respiratory syndrome *coronavirus*-2) in the biological material 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.

**List of Changes Made in the Instruction Manual**

| VER | Location of changes | Essence of changes |
|---|---|---|
| 28.04.14 ME | 4. Additional requirements | Mx3000P instrument was deleted |
| | 6. Sampling and handling | Through the text the phrase "at no more than minus 16 °C" was changed to "at the temperature from minus 24 to minus 16 °C" |
| 13.03.19 EM | 3. Content | The colour of the reagent was specified |
| 25.05.20 EM | Through the text | Information about a new type of coronavirus SARS-*Cov*-2 was added; all sections were updated according to the template; the text formatting was changed |
| | Footer | The phrase "Not for use in the Russian Federation" was added |
| | 2. Principle of PCR detection | The table with targets was added |
| 26.05.20 EM | 14. References | The name of Guidelines was specified |
| | 2. Principle of PCR detection | The table with targets was specified |
| 18.03.21 VA | — | The name, address and contact information for Authorized representative in the European Community was changed |
| 04.05.21 EM | Through the text | Environmental samples were added as a new type of the test material |

[5] The samples containing MERS-*Cov* and SARS-*Cov*-like viruses (SARS-*Cov*, SARS-*Cov*-2) are model samples of biological material containing recombinant quality control samples.
[6] The samples not containing MERS-*Cov* and SARS-*Cov*-like viruses (SARS-*Cov*, SARS-*Cov*-2) are samples of biological material from patients with ARVI, containing *influenza virus* A/H1N1pdm2009, *parainfluenza viruses*, *rhinoviruses*, and that has been proven in testing with reagent kits AmpliSens® *Influenza virus* A/B-FRT, AmpliSens® *Influenza virus* A/H1-swine-FRT and AmpliSens® ARVI-screen-FRT.
[7] Environmental samples were obtained from Reference Center for Monitoring of Acute Intestinal Infections by Rospotrebnadzor.

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