CE

IVD

For Professional Use Only

AmpliSens® SARS-*Coronavirus*-EPh

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® SARS-*Coronavirus*-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of SARS-*Coronavirus* RNA, which causes Severe Acute Respiratory Syndrome, in the clinical material (nasal, throat washes and swabs; feces; blood plasma) by using electrophoretic detection of the amplified products in agarose gel.

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

2. PRINCIPLE OF PCR DETECTION

SARS-*Coronavirus* detection detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen cDNA specific region using special SARS-CoV primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® SARS-*Coronavirus*-EPh PCR kit** PCR kit is a qualitative test, which contains the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. **AmpliSens® SARS-*Coronavirus*-EPh PCR kit** PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer. Wax melting and reaction mix components occur only at 95 ºC.

3. CONTENT

AmpliSens® SARS-*Coronavirus*-EPh PCR kit is produced in 2 forms:

AmpliSens® SARS-*Coronavirus*-EPh PCR kit **form 1** is consist of RIBO-sorb variant SARS-CoV, REVERTA-L variant SARS-CoV, PCR kit variant 100 R (0.5-ml tubes), EPh variant 200, **REF** TV29-100-R0,5-CE.

AmpliSens® SARS-*Coronavirus*-EPh PCR **form 2** is consist of RIBO-sorb variant SARS-CoV, REVERTA-L variant SARS-CoV, PCR kit variant 100 R (0.2-ml tubes), EPh variant 200,

REF TV29-100-R0,2-CE.

RIBO-sorb reagents kit variant SARS-CoV includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
Lysis Solution	colorless clear liquid *	60	1 vial
Washing Solution 1	colorless clear liquid *	40	1 vial
Washing Solution 3	colorless clear liquid	100	1 vial
Washing Solution 4	colorless clear liquid	40	1 vial
Sorbent	white suspension	1.25	2 tubes
RNA-buffer	colorless clear liquid	0.5	10 tubes

RIBO-sorb variant SARS-CoV reagents kit is intended for 100 reactions, including controls.

REVERTA-L RT reagents kit variant SARS-CoV includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
RT-G-mix-1	colorless clear liquid	0.01	10 tubes
RT-mix-SARS-CoV	colorless clear liquid	0.07	10 tubes
Revertase (MMlv)	colorless clear liquid	0.06	1 tube
DNA-buffer	colorless clear liquid	1.2	2 tubes

REVERTA-L variant SARS-CoV RT reagents kit is intended for 120 reverse transcription reactions, including controls.

*The precipitation in crystal habit is possible during storage of Lysis Solution and Washing Solution 1 at 2-8 °C.

AmpliSens® SARS-*Coronavirus*-EPh PCR kit variant 100 R includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-R SARS-CoV ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.01	110 tubes of 0.5 or 0.2 ml
PCR-mix-2 red	red clear liquid	1.2	2 tubes
Mineral oil for PCR	colorless viscous liquid	4.0	1 dropper bottle
Positive Control cDNA SARS-CoV (C+ $_{SARS-CoV}$)	colorless clear liquid	0.4	1 tube
DNA-buffer	colorless, clear liquid	0.5	2 tubes
Negative Control (C-)*	straw-colored clear liquid	1.6	6 tubes
Positive Control SARS-CoV-rec	colorless clear liquid	0.03	10 tubes
Internal Control SARS-CoV-rec (IC)	colorless clear liquid	0.06	10 tubes

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

AmpliSens® SARS-*Coronavirus*-EPh PCR kit variant 100 R is intended for 110 reactions, including controls.

EPh detection agarose kit variant 200 includes:

Reagent	*Description*	*Volume (ml), Mass (g)*	*Quantity*
Tris-borate buffer (TBE) concentrated with ethidium bromide	orange clear liquid	50 ml	1 vial
Agarose for DNA electrophoresis	white powder	1.7 g	2 vials

EPh detection agarose kit variant 200 is intended for 240 samples (100 ml of gel is for 5 lines x 24 holes).

4. ADDITIONAL REQUIREMENTS

<u>For use in the Extraction Area:</u>

- Laminar box
- Thermostatic bath or dry block for tubes with controlled temperature and capability to incubate at 25-100 °C.
- Vacuum aspirator with flask for removing supernatant
- Desktop microcentrifuge with rotor for 2 ml reaction tubes (RCF max. 16,000 x g)
- Vortex mixer
- Pipettes (adjustable)
- Disposable 1.5 ml volume polypropylene sterile screw-on or tightly closing tubes

- Tube racks
- Sterile pipette tips with aerosol barriers to 200 µl and 1000 µl
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Disposable powder-free gloves and laboratory coat
- Container with disinfectant

For use in the Reverse Transcription and Amplification Area:

- Personal thermocyclers (for example “GeneAmp PCR System 2700” (“Applied Biosystems”, USA) or equivalent).
- PCR box
- Vortex mixer
- Thermostatic bath or dry block for tubes with controlled temperature and capability to incubate at 25-100 °C.
- Pipettes (adjustable)
- Disposable sterile pipette tips with aerosol barriers (up to 200 µl)
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity
- Tube racks
- Disposable powder-free gloves and laboratory coat
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

For use in the Detection Area

- Horizontal electrophoresis chamber of not more than 400 ml volume
- Constant-current source with 150-460 V voltage
- UV transilluminator with room for gel scanning
- Digital camera for results registration and image transmission
- Distiller for water
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Microwave oven for agarose melting
- Conical heat-proof flask of 250 ml volume for agarose melting
- Graduated cylinder of 1 litre volume
- Tube rack
- Pipettes (adjustable)

- Pipette tips (up to 200 µl)
- Plastic container for deactivation of buffers and gels that contains ethidium bromide.
- Disposable powder-free gloves and laboratory coat

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 and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one directional, it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.

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


Xn

Lysis Solution,
Washing Solution 1

Contains guanidine thiocyonate. Guanidine thiocyonate is harmful if inhaled, or comes in contact with skin or if swallowed. Contact with acid releases toxic gas.
Harmful (Xn).
Risk and safety phrases:* R20/21/22-32, S13-26-36-46

Washing Solution 3,
Washing Solution 4

Contains ethanol: flammable. Risk phrase:* R10

*R10: Flammable;
R20/21/22: Harmful by inhalation, in contact with skin and if swallowed;
R32: Contact with acids liberates very toxic gas;
R36/37/38: Irritating to eyes, respiratory system and skin;
R42/43: May cause sensitization by inhalation and skin contact;
S13: Keep away from food, drink and animal feedingstuffs;
S22: Do not breathe dust;
S23: Do not breathe spray;
S24: Avoid contact with skin;
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice;
S36: Wear suitable protective clothing;
S36/37: Wear suitable protective clothing and gloves;
S46: If swallowed, seek medical advice immediately and show the container or label.


Xn

Tris-borate buffer (TBE) concentrated with ethidium bromide

Contains ethidium bromide: harmful.
Risk and safety phrases*
R22-26-36/37/38-68;
S26-28-36/37/39-45

*R22: Harmful if swallowed
R26: Very toxic by inhalation
R36/37/38: Irritating to the eyes, respiratory system and skin
R68: Possible risk of irreversible effects
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28: After contact with skin, wash immediately with plenty of water
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection
S45: In case of accident or if you feel unwell, seek medical advice immediately (show label where possible)

6. SAMPLING AND HANDLING

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

AmpliSens® SARS-*Coronavirus*-EPh PCR kit is intended for analysis of RNA extracted by RNA extraction kits from the following clinical material

- *Nasal, throat washes*
- *Nasal, throat swabs*
- *Feces*
- *Blood plasma*

6.1. *Nasal wash.* Sampling is carried out when patient sits with tilted back head. For

obtaining nasal wash input 3-5 ml of warm sterile saline into each nostril by using of disposable probe or syringe. Washed liquid from both nostrils is gathered into a single sterile tube by using funnel. Do not recycle the funnel without previous autoclaving.

6.2. *Throat wash.* Previous rinsing of mouth by water is needed before sampling. Then thoroughly rinsing of throat by 8-10 ml of saline solution during 10-15 s is provided. Liquid is gathered into a single sterile tube by using funnel. Do not recycle the funnel without previous autoclaving.

6.3. *Nasal swabs* are obtained by sterile cotton tampon. It is to be gently inserted into the nostril along the superior nose wall to the deep of 2-3 cm up to the inferior nasal concha. The tampon is to be gone down slightly, inserted into inferior nasal meatus under inferior nasal concha. Then the circular movement is to be done and the tampon is to be removed from the nose along superior nose wall. After sampling the working part of a probe with cotton tampon is to be placed into the tube with transport medium. Then it's to be rotated during 10-15 s trying to avoid the solution splashing. After it the probe is to be removed from the solution and the tube is to be closed.

6.4. *Throat swabs* are obtained by sterile cotton tampon with help of circular movements. Before sampling a patient is asked to rinse the mouth with water. Swabs are obtained from tonsillar area, palatine arches, and posterior oropharyngeal surface. After it the probe is to be removed from the solution and the tube is to be closed.

6.5. *Feces* are obtained from a sterile disinfected bedpan or a chamber-pot. Near 1.0 g is transferred into a sterile container of 60 ml volume by using of individual tips with aerosol barriers.

6.5.1 *Preparation of 10-20% fecal suspension* (for liquid feces).

1. Take 1.5 ml tubes with tightly sealed cap. Add 800 µl of phosphate buffer or sterile saline solution.
2. Transfer 0.1 g (100 µl) of fecal sample by individual spatulas into prepared tubes. Resuspend well to ensure homogenous suspension.

If a long-time storage it is necessary to add glycerin (up to final concentration of 10-15%), homogenize, incubate at room temperature for 30-40 min and frozen.

6.5.2. *Preparation clarified fecal extract.*

1. Spin the tube with prepared suspension or liquid feces at 10,000 g (12,000 rpm) for 5 min.
2. Remove 100 µl of supernatant, mix with 100 µl of Negative Control (C-), and use for RNA extraction. If a long-time storage is necessary then transfer supernatant in a disposable tube and frozen.

6.6. *Blood plasma.* Blood is taken fasting from ulnar veins by disposable [single-use] syringe or with help of Venoject or Vacuette® (lavender caps - 6% EDTA). Blood is transferred into disposable plastic tube with anticoagulant (6% solution of EDTA in ratio of 1:20 or 3.8% solution of sodium citrate in ratio of 1:9).

Do not use heparin as an anticoagulant.

Tube is to be closed and mixed carefully with anticoagulant. Blood plasma is obtained by tubes centrifuging at 800-1600 g (3,000 rpm) during 20 min at room temperature. Not less than 1 ml of plasma is sampled by using of individual tips with aerosol barriers and then transferred into 1.5 ml volume tubes. 200 µl of plasma sample is used for RNA extraction.

Only one freeze-thaw cycle of clinical material is allowed.

7. WORKING CONDITIONS

AmpliSens® SARS-*Coronavirus*-EPh PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA Extraction

1. **Lysis Solution** and **Washing Solution 1** (if stored at 2-8 ºC) should be heated at 60–65 ºC until the complete ice crystals dissolution.
2. Prepare the required number of 1.5 ml disposable polypropylene micro centrifuge tubes including one tube for Negative Control of Extraction (**Negative Control**, **C-**) and one tube for Positive Control of Extraction (**Positive Control, PCE**).
3. Add **5 µl** of **Internal Control SARS-CoV-rec** to each tube and then add **600 µl** of **Lysis Solution**. Mark the test tubes.
4. In case of blood plasma, nasal or throat swabs being analyzed add **200 µl** of sample into the tubes with **Lysis Solution** and **Internal Control SARS-CoV-rec** by using pipette tips with aerosol barriers.
5. In case of faeces, nasal or throat washes being analyzed add **100 µl** of **Negative Control** and **100 µl** of sample into the tubes with **Lysis Solution** and **Internal Control SARS-CoV-rec** by using pipette tips with aerosol barriers.
6. Prepare Controls as follows:
   6.1. Add **200 µl** of **Negative Control** to the tube labeled **C-**.
   6.2. Add **180 µl** of **Negative Control** and **20 µl** of **Positive Control SARS-CoV-rec** to the tube labeled **PCE**.
7. Tightly close all tubes and mix carefully on vortex for 7-10 s.

8. Centrifuge all tubes for 5 s at 5000g (for removing drops from internal surface of the lids).
9. Thoroughly resuspend **Sorbent** on vortex and add **25 µl** of it into each test tube.
10. Vortex tubes for 5 s, place in a rack for 60 s, once again mix on vortex for 5 s and incubate all tubes for 5 min at room temperature.
11. Centrifuge all tubes for 60 s at 10,000 g (for sorbent precipitation) and carefully remove supernatant from every tube by using vacuum aspirator. Use a new tip for every tube.
12. Add **400 µl** of **Washing Solution 1** into each tube. Vortex vigorously (until sorbent is fully resuspended), centrifuge for 60 s at 10,000 g. and carefully remove supernatant from every tube by using vacuum aspirator. Use a new tip for every tube.
13. Add **500 µl** of **Washing Solution 3** to each tube. Mix by Vortex vigorously and centrifuge for 60 s at 10,000g. Carefully remove supernatant from each tube by using vacuum aspirator. Use a new tip for every tube.
14. Repeat step 14.
15. Add **400 µl** of **Washing Solution 4** to each tube. Mix by Vortex vigorously and centrifuge for 60 s at 10,000g. Carefully remove supernatant from each tube by using vacuum aspirator. Use a new tip for every tube.
16. Incubate all tubes with open caps for 10-15 min at 60 °C (for sorbent predrying).
17. Add **50 µl** of **RNA-buffer** into tubes by using tip with aerosol barrier (RNAses-free)**.** Mix on vortex vigorously. Incubate for 5 min at 60 °C.
18. Once again mix on vortex and centrifuge the tubes for 2 min at maximum speed (12,000-16,000 g).

The supernatant contains purified RNA. The samples are ready to be used in reverse transcription reaction and PCR amplification.

It is recommended to carry out the reverse transcription reaction immediately after the RNA extraction. Carefully collect the solution of RNA for reaction without taking sorbent. If solution is muddy, centrifuge the tube to precipitate the sorbent.

The purified RNA can be stored:

- at 2-8 °C for 4 hours;
- at not more than minus 68 °C for 1 year (carefully collect the solution of RNA for reaction without taking sorbent).

8.2. Reverse transcription

Total reaction volume – **20 µl**, volume of RNA sample - **15 µl**.

1. Prepare required quantity of 0.2 (0.5) ml disposable polypropylene micro centrifuge

tubes.

2. Prepare reagent mix for 12 reactions as follows:
   - Add **5 µl** of **RT-G-mix-1** and **65 µl** of **RT-mix-SARS-CoV** into the tube carefully mix by vortex, centrifuge for 5-7 s (for removing drops from the internal surface of the test tubes caps).
   - Add **6 µl** of **Revertase (MMlv)** into the tube with reagent mix, then pipette 5 times and mix on vortex, then centrifuge for 5-7 s (for removing the drop with internal surface of the test tubes caps).
3. Transfer **5 µl** of reagent mix to each prepared test tube.
4. Add **15 µl** of **RNA-samples** into the tubes with reagent mix. Carefully mix by using the pipette (10 times).
5. Place the test tubes into thermocycler and incubate at for 30 minutes 37 °C.
6. Dilute obtained cDNA sample with DNA-buffer in proportion 1:2. For it add **20 µl** of DNA-buffer into each tube with **20 µl** of cDNA. Carefully mix by using the pipette (10 times).

cDNA samples can be stored:

- at not more than minus 16 °C for 4 week;
- at not more than minus 68 °C for 1 year.

8.3. Preparing the PCR

Total reaction volume - 50 µl, volume of cDNA sample - 20 µl.

8.3.1 Preparing tubes for PCR

1. Collect the required quantity of tubes with **PCR-mix-1-R SARS-CoV** with wax for amplification of cDNA of study and control samples.
2. Add **20 µl of PCR-mix-2 red** to the surface of wax layer, so that it wouldn't fall under the wax and mix with PCR-mix-1-R SARS-CoV.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl). When using thermocycler with heating cover this step could be omitted.

8.3.2 Amplification

Use prepared tubes for PCR. Under or immediately above the level of oil, using tips with aerosol barrier, **add 20 µl of cDNA samples**, obtained from clinical or control samples at the stage of reverse transcription.

Carry out the **control amplification reactions:**

NCA -Add 20 µl of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).
C+ -Add 20 µl of **Positive Control cDNA SARS-CoV** to the tube for Positive Control of Amplification.

Run the following program on the thermocycler (see table 1). When the temperature reaches 95 ºC (pause regimen), insert tubes to cells of amplifier and press button to continue.

It is recommended to sediment drops from walls of tubes by short vortex (1–3 s) before their insertion in thermocycler.

Table 1

Programming thermocyclers at cDNA amplification of SARS-*Coronavirus*

	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	GeneAmp PCR System 2400 (Applied Biosystems)			**GeneAmp PCR System 2700 (Applied Biosystems)**			**Biometra**		
step	temperature	time	cycles	temperature	time	cycles	temperature	time	cycles
1	**95 °C**	pause		**93 °C**	pause		**95 °C**	pause	
2	**95 °C**	5 min	1	**93 °C**	5 min	1	**95 °C**	5 min	1
3	**95 °C**	10 s	42	**93 °C**	10 s	42	**95 °C**	30 s	42
	63 °C	20 s		**65 °C**	40 s		**63 °C**	45 s	
	72 °C	20 s					**72 °C**	45 s	
4	**72 °C**	2 min	1	**72 °C**	2 min	1	**72 °C**	2 min	1
5	**10 °C**	storage		**4 °C**	storage		**10 °C**	storage	

Amplification in thermocycler with block temperature adjustment lasts 2 h, in thermocycler with active temperature adjustment — 1 h 30 min.

After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis.

Analysis of amplification products is performed by separation of cDNA fragments in agarose gel.

The amplified samples can be stored for 16 h at room temperature, for 1 week at 2–8 °C (be sure to warm the samples to room temperature before running electrophoresis).

9. DATA ANALYSIS

9.1. Preparation of working solutions and agarose gel

9.1.1. Buffer for electrophoresis

Add **25 ml** of **Tris-borate buffer (TBE) concentrated with ethidium bromide** into the graduated cylinder. Then add **distilled water** to the volume of **500 ml**, close the cylinder by parafilm and mix.

Ethidium bromide is carcinogenic substance. Use it in compliance with general precautions. All reagents contained ethidium bromide should be utilized in compliance with local authorities' requirements.

9.1.2. Agarose gel

1. Transfer 1.7 g of agarose powder into the heat-proof flask of 250 ml volume. Then add 100 ml of prepared buffer, stir, and melt in microwave oven until agarose is completely dissolve. Agarose melting time is 1.5 min in 800W microwave oven if loaded with 1

flask (or 5 min - if 5 flasks are loaded).

2. Take out the flask with melted agarose from microwave and mix carefully by rotating. Then place the flask into the microwave oven for 1.5 min (800W) and boil agarose. Take out the flask from the microwave oven and cool to 65-70 °C by rotating.
3. Level the table for filling with gel. Fill up the melted gel into the form of horizontal electrophoresis chamber. Insert combs into the gel without touching of the form bottom. The distance between combs should be not less than 3 cm from each other. Gel thickness must be about 6 mm.
4. After the gel has completely thickened (about 30 minutes at room temperature), remove the combs carefully without damaging of the holes. Transfer the padding with prepared gel into the horizontal electrophoresis chamber. Holes are to be placed nearer to the negative electrode (DNA will move to the positive electrode). Add prepared buffer into the chamber ensuring it covers gel by 5 mm above.

9.2. Procedure

1. Tubes with amplification's products are to be placed on the tube racks. Take **10-15 µl** of amplified DNA samples from under the oil and place into gel holes (if you use one tip for different samples then you need to wash it by buffer after each sampling).

Molecular weight marker (not supplied with EPh detection kit) and Positive Control (C+) should be present in each holes line.

2. Turn on power supply to the chamber keeping the polarity (DNA moves to the positive electrode) and switch the power supply on. If using SE-2 (Helicon) chamber for electrophoresis and Elf-4 (DNA-Technology) power supply apply following parameters: voltage - 250 V, voltage stabilization, electrophoresis time - 18-20 min. Optimum electric-field strength is 10 V/cm in such conditions.
3. When dye reaches approximately 2/3 of the gel length (2 cm) switch current source off. Transfer the gel on the transilluminator, placing strips horizontally with holes up. Get the gel image on the computer by using of video system, register the samples order and add the image to database.

Put the protective mask or use the glass filter while watching and photographing the gel.

9.3. Results interpretation.

Analysis of results is based on the presence or absence of specific bands of amplified cDNA in agarose gel (1.7%). The length of specific amplified cDNA fragments is:

- SARS-*Coronavirus* – 221 bp
- IC SARS-CoV-rec – 400 bp

Table 2

Results for controls

Control	Controlled step	Specific bands in the agarose gel		Interpretation
		221 bp	400 bp	
PCE	RNA extraction	**Yes**	**Yes**	OK
C-	RNA extraction	No	**Yes**	OK
NCA	Amplification	No	No	OK
C+	Amplification	**Yes**	No	OK

- The sample is considered to be positive for SARS-CoV RNA if the band of 221 bp is present in agarose gel. The band of IC (400 bp) could be absent in the samples with high concentration of SARS-*Coronavirus* RNA.
- The sample is considered to be negative for SARS-*Coronavirus* RNA if the band of 221 bp is absent and the band of 400 bp is present.

Besides specific bands the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 bp of nucleotide pairs.

10. TROUBLESHOOTING

Results of analysis are not being registered in the following cases:

- If results of control points analysis do not correspond to the listed above (Table 2), then the tests are to be re-installed. Discard any reagents that may be suspect.
- If in lanes none of bands of 221 and 400 nucleotide pairs is observed, result of analysis for this sample is irrelevant and investigation of this sample must be repeated from the very beginning. It can be caused by mistake in clinical processing that provoked loss of RNA/DNA or inhibition of RT and/or PCR.
- If in lines nonspecific bands at different levels are presented, it may be caused by lack of “hot start” or false temperature regimen in thermocycler.
- If in lanes corresponding to negative control (NCA, C–) specific band of 221 bp appears, it means that reagents or samples contamination has taken place. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting contamination source must be undertaken.

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

11. TRANSPORTATION

AmpliSens® SARS-*Coronavirus*-EPh PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® SARS-*Coronavirus*-EPh** PCR kit (except of all components of the REVERTA-L variant SARS-CoV RT reagents kit and all components of the EPh detection agarose kit variant 200) are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® SARS-*Coronavirus*-EPh** PCR kit are to be stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

All components of the REVERTA-L variant SARS-CoV RT reagents kit are to be stored at temperature from minus 24 to minus 16 ºC when not in use.

All components of the EPh detection agarose kit variant 200 are to be stored at 18-25 ºC when not in use. All components of the EPh detection agarose kit variant 200 are to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® SARS-*Coronavirus*-EPh** PCR kit is not less than $5x10^3$ genome equivalents of SARS-*Coronavirus* RNA per 1.0 ml of blood plasma, nasal and throat swabs and washes and not less than $1x10^4$ genome equivalents of SARS-*Coronavirus* RNA per 1.0 ml of feces.

The claimed analytical features of AmpliSens® SARS-*Coronavirus*-EPh PCR kit are guaranteed only when the whole kit of reagents is used.

13.2. Specificity

Specificity of **AmpliSens® SARS-*Coronavirus*-EPh** PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.

14. REFERENCES

1. Manual “Sampling, transportation and storage of clinical material for PCR diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”, Moscow, 2008.

15. QUALITY CONTROL

In accordance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® SARS-*Coronavirus*-EPh** PCR kit is tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Expiration Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version	**PCE**	Positive control of extraction
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	Positive control of amplification
EC REP	Authorised representative in the European Community	**IC**	Internal control
	Flammable		Harmful (Xn)
	Caution		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
23.12.10 KM	Cover page	The phrase “For Professional Use Only” was added
	Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” was added.
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of open reagents was added
	Key to Symbols Used	The explanation of symbols was corrected
27.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
25.05.12 BO	13.1 Sensitivity	The value and units of analytical sensitivity were corrected