AmpliSens® COVID-19-FL PCR kit

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

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

1. INTENDED USE

AmpliSens® COVID-19-FL PCR kit is an *in vitro* nucleic acid amplification test for detection and quantitative determination of SARS-CoV-2 RNA in the biological material (nasopharyngeal and oropharyngeal swabs, sputum / pharyngeal aspirate, bronchoalveolar lavage / bronchial washing fluids, blood plasma, fecal / rectal swab, autopsy material) and in the environmental samples (water sample concentrates, washes from environmental objects) using real-time hybridization-fluorescence detection of amplified products. The material for RT-PCR is RNA samples extracted from biological material.

Indications and contra-indications for use of the reagent kit

The reagent kit is used for investigation of biological material taken from the persons who arrived from the regions where COVID-19 cases are registered, persons who had contact with COVID-19 patients, without distinction of form and presence of manifestation, patients with acute respiratory infection with COVID-19 suspicion, for investigation of autopsy material of lungs in order to establish etiology of pneumonia, as well as for investigation of environmental samples in order to prevent coronavirus infection in humans.

There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

The RT-PCR result should be interpreted according to the clinical and epidemiological data. Negative RT-PCR result does not rule out the possibility of infection with SARS-CoV-2 coronavirus and can be obtained if the virus content in the sample is below the specified analytical sensitivity.

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-FL (IC)), simultaneous RNA reverse transcription and amplification of cDNA fragments of the detected microorganism and cDNA of the internal control with hybridization-fluorescence detection. Exogenous internal control (Internal Control-FL (IC)) allows to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition.

RNA reverse transcription with the revertase enzyme 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.

The quantitative analysis of SARS-CoV-2 RNA is based on the linear dependence between the logarithm of initial RNA target concentration in a test sample and exponential growth of fluorescence signal. PCR-analysis of test samples is carried out simultaneously with a sample with the known concentration of the RNA target – Positive Control, which is assigned according to the Manufacturer's measurement procedure. Calculation of RNA-target concentration in test samples is carried out according to the results of Positive Control amplification.

AmpliSens® COVID-19-FL 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 results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE
cDNA-target	IC cDNA	SARS-CoV-2 cDNA
Target gene	Artificially synthesized sequence	RdRp gene (RNA-dependent RNA polymerase)

3. CONTENT

AmpliSens® COVID-19-FL PCR kit is produced in 1 form:
variant FRT-100 F, REF H-4094-1-1-CE

Variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-FL SARS-CoV-2	clear liquid from colorless to light lilac colour	1.2	1 tube
PCR-buffer-C	colorless clear liquid	0.6	1 tube
Polymerase (TaqF)	colorless clear liquid	0.06	1 tube
Revertase-H	colorless clear liquid	0.03	1 tube
RT-G-mix-2	colorless clear liquid	0.03	1 tube
C+ SARS-CoV-2	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	4 tubes
Internal Control-FL (IC)*	colorless clear liquid	1.0	1 tube
Positive Control SARS-CoV-2*	colorless clear liquid	0.2	1 tube

* must be used in the extraction procedure.
Variant FRT-100 F is intended for 110 reactions (including controls).

AmpliSens® COVID-19 software (version 1.0).

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).
- Vacuum tubes for sampling, storage and transportation of blood samples.
- Flexible flocked or fiber swabs for collecting nasopharyngeal specimens.
- Sterile swab with viscose tip in individual package.
- Plastic container (50-60 ml) for storage and transportation of biological samples.
- Disposable tightly closed polypropylene 1.5-ml and 2.0-ml tubes for sampling and pretreatment.
- Sterile tools (individual for each sample) for homogenization (porcelain mortar and mallet) or homogenizer for pretreatment of tissue (autopsy) material.
- Sterile RNase-free pipette tips with aerosol filters (up to 10, 100, 200, 1000, 5000 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge up to 12,000 g (suitable for Eppendorf tubes).
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir to throw off and inactivate the material.
- Disposable powder-free gloves and a laboratory coat.

For RNA extraction, reverse transcription and amplification

- RNA extraction kit.
- Sterile RNase-free pipette tips with aerosol filters (up to 10, 100, 200 µl).
- Tube racks.
- Vortex mixer.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); 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 (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use 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 specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid 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 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

AmpliSens® COVID-19-FL PCR kit is intended for analysis of the RNA extracted with the use of RNA extraction kits from the biological material (nasopharyngeal and oropharyngeal swabs, sputum / pharyngeal aspirate, bronchoalveolar lavage / bronchial washing fluids, blood plasma, fecal / rectal swab, autopsy material) and environmental samples (water sample concentrates, washes from environmental objects).

It is recommended to investigate at least two types of biological material (material taken from the respiratory tract, blood plasma, and fecal / rectal swabs in the presence of symptoms of gastrointestinal tract disease).

The material used for the investigation:

- nasopharyngeal and oropharyngeal swabs (in the presence of symptoms of upper respiratory tract involvement),
- sputum / pharyngeal aspirate (in the presence of symptoms of lower respiratory tract involvement),
- bronchoalveolar lavage / bronchial washing fluids (in the presence of symptoms of lower respiratory tract involvement),
- blood plasma,
- fecal / rectal swab (in the presence of symptoms of gastrointestinal tract disease),
- autopsy material,
- water sample concentrates,
- washes from environmental objects.

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** and analyze them as a single sample.

Sampling

Nasopharyngeal and oropharyngeal swabs

Nasopharyngeal swabs. If the nasal cavity is full of mucus it is recommended to blow the nose before the procedure. Nasopharyngeal swabs are collected with sterile dry flocked swab with plastic shaft. 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**, the flexible part of the swab is folded up 3 times, 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**. Break off the end of shaft to allow tight closing of tube cap. Close and mark the tube with the solution and the swab.

Nasopharyngeal and oropharyngeal swabs can be stored before pretreatment:

- 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 acceptable.

Sputum / pharyngeal aspirate is collected into sterile hermetic disposable plastic containers. If it is impossible to collect sputum or pharyngeal aspirate, collect the saliva formed in the morning after deep coughing immediately after waking.

Sputum / pharyngeal aspirate can be stored before pretreatment:

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

Only one freeze-thawing cycle is acceptable.

Bronchoalveolar lavage / bronchial washing fluids are collected into disposable tightly screwed plastic containers with a volume no less than 5 ml.

Bronchoalveolar lavage / bronchial washing fluids can be stored before pretreatment:

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

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 the tube (vacuum system) with EDTA as anticoagulant. The closed tube is to be rotated gently several times for mixing with the anticoagulant and stored at the temperature from 2 to 8 °C.

The tube with whole blood is centrifuged at 800-1,600 rpm during 20 min at room temperature no later than 6 hours after blood collection. Then no less than 0.5 ml of blood plasma is taken with separate tips with aerosol filters into sterile 1.5-2.0-ml tubes. 50 µl of blood plasma is used for the RNA extraction.

Blood plasma 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 year;
- at the temperature not more than minus 68 °C – for a long time.

Only one freeze-thawing cycle is acceptable.

Fecal / rectal swab

Fecal swab is collected from a diaper or from the surface of a disposable bag placed in a pot or vessel. Place the polypropylene swab with viscose tip into the fecal sample, keep until soaked, then break off the viscose tip into the tube with 500 µl of transport medium (sterile saline solution or phosphate buffered saline) holding it with a tube lid and tightly closing the tube.

Rectal swab. Place the polypropylene swab with viscose tip in the rectum to a depth of 2 cm, rotate and remove it. Break off the viscose tip into the tube with 500 µl of transport medium (sterile saline solution or phosphate buffered saline) holding it with a tube lid and tightly closing the tube.

Fecal / rectal swab can be stored before pretreatment:

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

Only one freeze-thawing cycle is acceptable.

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

Autopsy material can be stored before pretreatment:

- at the temperature from minus 24 to minus 16 °C – for 1 year.

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 *blood plasma, water sample concentrates and washes from environmental objects* is not required.

Nasopharyngeal and oropharyngeal swabs. Vortex the tube for 5 s to sediment drops from the interior wall of the tube lid. If the liquid level in the tube is below 0.5 ml, the volume should be increased by adding 0.5 ml of saline solution, vortex the tube for 5 s to sediment drops from the interior wall of the tube lid. If there is mucus in the sample, **Mucolysin** reagent should be added up to the mark of 1 ml. Incubate at the room temperature (from 18 to 25 °C) for 5 min (until visual clarification). In case of sedimentation centrifuge the samples at 600 rpm for 5 min. 100 µl of sample is used for RNA extraction. The residual sample should be frozen if it is necessary to repeat the analysis.

Bronchoalveolar lavage / bronchial washing fluids. Mix the sample by pipetting using a tip with aerosol filter. 100 µl of suspension is used for RNA extraction.

Sputum / pharyngeal aspirate. Use **Mucolysin** reagent for viscous sputum pretreatment. In order to reduce the viscosity of sputum a fivefold amount of Mucolysin reagent should be added to the container with sputum and incubated at the room temperature (from 18 to 25 °C) for 10-20 min (until visual clarification). In case of sedimentation transfer the sample to a 1.5-ml tube and centrifuge at 600 rpm for 5 min. 100 µl of supernatant is used for RNA extraction. The residual sputum should be frozen if it is necessary to repeat the analysis.

Fecal / rectal swab. Vortex the tube, then centrifuge it at 600 rpm for 5 min. 50 µl of supernatant is used for RNA extraction.

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 6,700 rpm for 5 min. 100 µl of supernatant is used for RNA extraction. The residual suspension should be frozen if it is necessary to repeat the analysis.

The pretreated samples can be stored before the PCR-analysis:

- at the room temperature – for 6 hours;
- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for 1 year;
- at the temperature not more than minus 68 °C – for a long time.

Interfering substances and limitations of using test material samples

Whole blood samples collected in the tubes with heparin as anticoagulant are inapplicable for analysis.

In order to control the RNA extraction efficiency and possible reaction inhibition the Internal Control (Internal Control-FL (IC)) is used in the PCR kit. The Internal Control is added in each biological sample at the extraction stage. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.

Potential interfering substances

Endogenous and exogenous substances that may be present in the biological material (nasopharyngeal and oropharyngeal swabs, sputum / pharyngeal aspirate, bronchoalveolar lavage / bronchial washing fluids, blood plasma, fecal / rectal swab, autopsy material) used for the study were selected to assess potential interference.

Model samples of biological material without adding and with the addition of potential interfering substances were tested (see Table 2). Model samples contained quality control sample (QCS) with SARS-CoV-2 RNA concentration of $5x10^3$ GE/ml.

Table 2

Test material	Type of potential interferent	Potential interferent	Tested concentration in a sample	Interference presence	
				RIBO-prep	MAGNO-sorb
Nasopharyngeal and oropharyngeal swabs	Endogenous substances	Mucin	6 mg/ml	Not detected	Not tested
			9 mg/ml	Detected	Not detected
		Hemoglobin	0,21 g/ml	Not detected	Not detected
	Exogenous substances	Chlorhexidine aqueuos solution	2,5 %	Not detected	Not detected
Sputum	Endogenous substances	Mucin	6 mg/ml	Not detected	Not tested
			9 mg/ml	Detected	Not detected
Bronchoalveolar lavage / bronchial washing fluids	Endogenous substances	Mucin	6 mg/ml	Not detected	Not tested
			9 mg/ml	Detected	Not detected
		Hemoglobin	0,21 g/ml	Not detected	Not detected
Fecal / rectal swab	Endogenous substances	Mucin	6 mg/ml	Not detected	Not tested
			9 mg/ml	Detected	Not detected
		Hemoglobin	0,21 g/ml	Not detected	Not detected
Blood plasma	Endogenous substances	Hemoglobin	0,21 g/ml	Not detected	Not detected
Autopsy material	Endogenous substances	Hemoglobin	0,21 g/ml	Not detected	Not detected

7. WORKING CONDITIONS

AmpliSens® COVID-19-FL 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

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

– **RIBO-prep**;
– **MAGNO-sorb**.

The RNA extraction of each test sample is carried out in the presence of **Internal Control-FL (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 SARS-CoV-2** to the tube labeled PCE (Positive Control of Extraction)

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

NOTE: Washing Solution 5 is not used when carrying out the extraction with MAGNO-sorb

NOTE: RNA extraction is performed from 100 µl of prepared environmental samples and biological material except for fecal / rectal swabs and blood plasma. When working with fecal / rectal swabs and blood plasma, 50 µl of the biomaterial sample and 50 µl of Negative Control (C–) are used for RNA extraction.

It is recommended to carry out the RT-PCR just after obtaining the RNA samples. It is allowed to store the RNA samples at the temperature from 2 to 8 °C for 30 min, at the temperature from minus 24 to minus 16 °C for 1 week and at the temperature ≤ –68 °C for 1 year. Only one freeze-thawing cycle is required.

8.2. Preparing reverse transcription and PCR

8.2.1 Preparing tubes for RT-PCR

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

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

1. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:
   – **10 µl** of **PCR-mix-FL SARS-CoV-2**,
   – **5 µl** of **PCR-buffer-C**,
   – **0.5 µl** of **Polymerase (TaqF)**,
   – **0.25 µl** of **Revertase-H**,
   – **0.25 µl** of **RT-G-mix-2**.

Prepare the reaction mixture for the total number of test and control samples plus some extra reaction. See numbers of control samples in item 7.

The calculation for the required number of reactions including testing the test and control samples can be performed according to Table 3.

Table 3

Scheme of reaction mixture preparation

		Reagent volume for specified number of reactions				
Reagent volume per one reaction, µl		10.0	5.0	0.25	0.50	0.25
Number of test samples	Number of reactions[1]	PCR-mix-FL SARS-CoV-2	PCR-buffer-C	RT-G-mix-2	Polymerase (TaqF)	Revertase-H
1	4	40	20	1.00	2.00	1.00
2	5	50	25	1.25	2.50	1.25
3	6	60	30	1.50	3.00	1.50
4	7	70	35	1.75	3.50	1.75
5	8	80	40	2.00	4.00	2.00
6	9	90	45	2.25	4.50	2.25
7	10	100	50	2.50	5.00	2.50
8	11	110	55	2.75	5.50	2.75
9	12	120	60	3.00	6.00	3.00
10	13	130	65	3.25	6.50	3.25
11	14	140	70	3.50	7.00	3.50
12	15	150	75	3.75	7.50	3.75
13	16	160	80	4.00	8.00	4.00
14	17	170	85	4.25	8.50	4.25
15	18	180	90	4.50	9.00	4.50
16	19	190	95	4.75	9.50	4.75
17	20	200	100	5.00	10.00	5.00
18	21	210	105	5.25	10.50	5.25
19	22	220	110	5.50	11.00	5.50
20	23	230	115	5.75	11.50	5.75
21	24	240	120	6.00	12.00	6.00
22	25	250	125	6.25	12.50	6.25
23	26	260	130	6.50	13.00	6.50
24	27	270	135	6.75	13.50	6.75
25	28	280	140	7.00	14.00	7.00
26	29	290	145	7.25	14.50	7.25
27	30	300	150	7.50	15.00	7.50
28	31	310	155	7.75	15.50	7.75
29	32	320	160	8.00	16.00	8.00
30	33	330	165	8.25	16.50	8.25
31	34	340	170	8.50	17.00	8.50
32	35	350	175	8.75	17.50	8.75

NOTE: Prepare the reaction mixture just before use.

2. Thaw the tube with **PCR-mix-FL SARS-CoV-2.** Thoroughly vortex all the reagents of the PCR kit and sediment the drops by vortex.
3. In a new tube prepare the reaction mixture. Mix the required quantities of **PCR-mix-FL SARS-CoV-2, PCR-buffer-C**, **Polymerase (TaqF), Revertase-H** and **RT-G-mix-2**. Sediment the drops by vortex.
4. Take the required number of the tubes or strips for RT-PCR of RNA of test and control samples.
5. Transfer **15 µl** of the prepared reaction mixture to each tube. Discard the unused reaction mixture.
6. Using tips with aerosol filter, add **10 µl** of **RNA samples** obtained at the RNA extraction stage.

   NOTE: Avoid transferring the sorbent together with the RNA samples extracted by MAGNO-sorb variant 100.

7. Carry out the control reactions:

   PCE – Add **10 µl** of **the sample extracted from the Positive Control** to the tube labeled PCE (Positive Control of Extraction).

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

NOTE: It is necessary to carry out the control reactions of RT-PCR (C+, NCA) for a new lot of reagent kit.

To check the amplifiers functioning and in other cases of internal laboratory control, carry out an additional control reaction for Positive Control of Amplification:

C+ – Add **10 µl** of **C+ SARS-CoV-2** to the tube labelled C+ (Positive Control of Amplification).

To rule out possible contamination, carry out an additional control reaction for Negative Control of Amplification:

NCA – Add **10 µl** of **TE-buffer** to the tube labelled NCA (Negative Control of Amplification).

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

NOTE: Carry out RT-PCR just after the mix of reaction mixture and RNA-samples and controls.

8.2.2. Amplification

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

Table 4

Amplification program

Step	Rotor-type instruments[2]			Plate-type instruments[3]		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
1	50	20 min	1	50	20 min	1
2	95	15 min	1	95	15 min	1
3	95	10 s	5	95	10 s	5
	60	20 s		60	20 s	
4	95	10 s	40	95	10 s	40
	60	20 s Fluorescence acquiring		60	20 s Fluorescence acquiring	

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

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin*.
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.
   Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type 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, using the algorithm below or by AmpliSens® COVID-19 software version 1.0.

The curves of fluorescent signal accumulation indicating the amplification product accumulation are analyzed in two channels:

Table 5

Channel for the fluorophore	FAM	JOE
Amplification product	Internal Control-FL (IC) cDNA	SARS-CoV-2 cDNA

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 RNA sample in the corresponding column of the results grid.

Calculation of RNA-target concentration in the test sample is carried out for quantitative analysis according to *Ct* values obtained for the test sample and Positive Control.

Principle of results interpretation for qualitative analysis is the following:

Table 6

Results Interpretation for the test samples when performing qualitative analysis

Ct value in the channel for the fluorophore		Result
FAM	JOE	
< boundary value	absent	SARS-CoV-2 RNA **is not detected**
determined or absent	< boundary value	SARS-CoV-2 RNA **is detected**
absent or > boundary value	absent	**Invalid***
determined or absent	> boundary value	**Equivocal****

* In case of **invalid** result, the PCR analysis should be repeated for the corresponding test sample starting from the RNA extraction stage.

** In case of **equivocal** result it is necessary to repeat PCR analysis for the corresponding test sample, starting from the RNA extraction stage. If the same result was obtained once again, the sample is considered equivocal and re-sampling of the material for analysis is recommended.

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

Principle of results interpretation for quantitative analysis is the following:

The samples with result "SARS-CoV-2 RNA is detected" in qualitative analysis can be interpreted in quantitative format.

The calculation is based on the formula:

$$C_S = C_{PC} \times 2^{(Ctpc - Cts)},$$

where

C_S – concentration of SARS-CoV-2 RNA in the test sample in GE (copies) / ml;

C_{PC} – concentration of Positive Control specified in the *Important Product Information Bulletin* enclosed to the PCR kit;

Ct_{PC} – *Ct* value of Positive Control of Extraction in the channel for the JOE fluorophore;

Ct_S – *Ct* value of sample in the channel for the JOE fluorophore.

[1] Number of reactions including the number of test samples (N) and the controls of extraction stage plus one extra reaction (N+2+1). If the controls of RT-PCR (C+, NCA) are used, the number of reactions will increase by 2.

[2] For example, Rotor-Gene Q (QIAGEN, Germany).

[3] For example, CFX 96 (Bio-Rad, USA).

The result of quantitative analysis (SARS-CoV-2 RNA concentration in the test sample) can be additionally interpreted according to arbitrary scale:

Table 7

Arbitrary scale of results interpretation for quantitative analysis

Concentration value, GE (copies) / ml	Result interpretation in accordance with arbitrary scale
$C_S \geq 10^8$	**Very high load** of SARS-CoV-2 RNA
$10^6 \leq C_S < 10^8$	**High load** of SARS-CoV-2 RNA
$5x10^4 \leq C_S < 10^6$	**Average load** of SARS-CoV-2 RNA
$10^3 \leq C_S < 5x10^4$	**Low load** of SARS-CoV-2 RNA
$C_S < 10^3$	**Very low load** of SARS-CoV-2 RNA*

* Only applicable to the following test material: nasopharyngeal and oropharyngeal swabs, bronchoalveolar lavage / bronchial washing fluids, water sample concentrates, washes from environmental objects.

Note – According to the literature data, SARS-CoV-2 RNA concentration in biological material from infected individuals (nasopharyngeal and oropharyngeal swabs, sputum, feces) varies from ~500 GE (copies)/ml (limit of detection) to $1x10^{10}$ GE (copies)/ml [1, 2]. Average value of SARS-CoV-2 RNA concentration in sputum of patients with pneumonia is $1.1x10^5$ GE (copies)/ml (CI 95% ($3.6x10^4$ - $3.3x10^5$ GE (copies)/ml)), in nasopharyngeal and oropharyngeal swabs – $4.3x10^4$ GE (copies)/ml (CI 95% ($2.0x10^4$ – $9.4x10^4$ GE (copies)/ml)) [1]. SARS-CoV-2 RNA median concentration in nasopharyngeal and oropharyngeal swabs from children infected with SARS-CoV-2 is $3.6×10^7$ GE (copies)/ml, in feces – $4.8×10^7$ GE (copies)/ml [2].

Quality control of analysis

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of extraction and RT-PCR are correct (see Table 8).

NOTE: Quantitative analysis is carried out only when the cycle threshold (*Ct*) values for Positive Control of extraction (PCE) and test samples are less than the boundary values.

Table 8

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM	JOE
PCE	RNA extraction	< boundary value	< boundary value
C–	RNA extraction	< boundary value	absent
NCA	RT-PCR	absent	absent
C+	RT-PCR	< boundary value	< boundary value

Quality control of quantitative analysis can be performed by testing an additional repeat of PCE (Positive Control SARS-CoV-2) identified as a quantitative analysis quality control (QAQC). The obtained concentration for QAQC sample should fall within the range specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

10. TROUBLESHOOTING

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

1. The *Ct* value determined for the Positive Control of reverse transcription and amplification (C+) in any of the channels for the FAM and/or JOE fluorophores is greater than the boundary value or absent. The amplification should be repeated for all the samples in which the specific RNA was not detected.
2. The *Ct* value determined for the Positive Control of Extraction (PCE) in the channels for the FAM and/or JOE fluorophores is greater than the boundary value or absent. The PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples.
3. If the *Ct* value is determined for the Negative Control of Extraction (C–) in the channel for JOE fluorophore. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples in which specific RNA was detected.
4. If the *Ct* value is determined for the Negative Control of reverse transcription and amplification (NCA) in the channels for the FAM and/or JOE fluorophores. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The amplification and detection should be repeated for all samples in which specific RNA was detected.
5. The *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level or parameters of base line calculation. If the result has been obtained with the correct level of threshold line (base line), the amplification and detection should be repeated for this sample.

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

11. TRANSPORTATION

AmpliSens® COVID-19-FL PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® COVID-19-FL** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-FL SARS-CoV-2, PCR-buffer-C, polymerase (TaqF), revertase-H and RT-G-mix-2). All components of the **AmpliSens® COVID-19-FL** 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 SARS-CoV-2, PCR-buffer-C, polymerase (TaqF), revertase-H and RT-G-mix-2 are to be stored at the temperature from minus 24 to minus 16 °C

NOTE: PCR-mix-FL SARS-CoV-2 is to be kept away from light

13. SPECIFICATIONS

13.1. Measurement range and limit of detection

Table 9

Test material	Nucleic acid extraction kit	PCR kit	Limit of detection, GE (copies)/ml	Measurement range, GE (copies)/ml
Nasopharyngeal and oropharyngeal swabs	RIBO-prep, MAGNO-sorb	variant FRT-100 F	$5x10^2$	$5x10^2$ – $3x10^8$
Sputum / pharyngeal aspirate			$1x10^3$	$5x10^3$ – $3x10^8$
Bronchoalveolar lavage / bronchial washing fluids			$5x10^2$	$5x10^2$ – $3x10^8$
Blood plasma			$1x10^3$	$1x10^4$ – $3x10^8$
Fecal / rectal swab			$1x10^3$	$1x10^4$ – $3x10^8$
Autopsy material			$1x10^3$	$5x10^3$ – $3x10^8$

Note – one copy of cDNA target corresponds to one genomic equivalent (genome, GE) of the microorganism.

The claimed features are achieved while respecting the rules specified in the section "*Sampling and Handling*".

13.2. Analytical specificity

The analytical specificity of **AmpliSens® COVID-19-FL** PCR kit is ensured by the 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 reagent kit detects a fragment of SARS-CoV-2 RNA (clinical samples with SARS-CoV-2 RNA in concentration from $5x10^2$ to $5x10^6$ GE (copies)/ml, the specificity was confirmed by direct sequencing of nucleotide sequences. The analytical specificity was proved when investigating the RNA/DNA of the following microorganisms/strains:

- strains from ATCC (American Type Culture Collection, USA): *Streptococcus pneumoniae* (ATCC® 49619™), *Staphylococcus aureus* subsp. *aureus*, Strain Seattle 1945 (ATCC® 25923™), *Pseudomonas aeruginosa* (ATCC® 9027™), *Moraxella catarrhalis* (ATCC® 8193™), *Neisseria mucosa* (ATCC® 19693™), *Enterococcus faecalis* (ATCC® 19433™), *Mycoplasma pneumoniae*, Strain PI 1428 (ATCC® 29085™), *Chlamydophila pneumoniae*, Strain CM-1 (ATCC® VR-1360™), *Legionella pneumophila* subsp. *pneumophila*, Strain Philadelphia 1 (ATCC® 33152™), *Staphylococcus epidermidis*, FDA Strain PCI 1200 (ATCC® 12228™), *Bacillus cereus*, Strain FDA 5 (ATCC® 10702™), *Human Respiratory Syncytial Virus*, Strain 9320 (ATCC® VR-955™), *Human Respiratory Syncytial Virus*, Strain A-2 (ATCC® VR-1540™), *Human Parainfluenza Virus* 1, Strain C35 (ATCC® VR-94™), *Human Parainfluenza Virus* 2, Strain Greer (ATCC® VR-92™), *Human Parainfluenza Virus* 3, Strain C243 (ATCC® VR-93™), *Human Rhinovirus* 17, Strain 33342 (ATCC® VR-1663™), *Human Adenovirus* 1, Strain Adenoid 71 (ATCC® VR-1™), *Human Coronavirus*, Strain OC43 (ATCC® VR-1558™), *Human Coronavirus*, Strain 229E (ATCC® VR-740™), *Human Coxsackievirus* B1, Strain Conn-5 (ATCC® VR-28™), *Human Echovirus* 4, Strain Pesascek (ATCC® VR-1734™), *Human Herpesvirus* 1, Strain HF (ATCC® VR-260™), in concentration no more than $1x10^9$ GE/ml and no less than $1x10^6$ GE/ml;
- strains of SCPM collection (State Collection of Pathogenic Microorganisms) *Haemophilus influenzae* 423, *Streptococcus pyogenes* Dick – I, *Corynebacterium pseudodiphtheriticum* №25, *Proteus mirabilis* 3177, *Klebsiella pneumoniae* 418, *Escherichia coli* M 17, *Salmonella typhimurium* 79, *Yersinia enterocolitica* 134, *Bordetella pertussis* 703 L 6, *Mycobacterium Bovis Ravenel* №700204 in concentration no more than $1x10^9$ GE/ml and no less than $1x10^6$ GE/ml;
- samples of the panel «CORONAVIRUS RNA SPECIFICITY PANEL» (Erasmus Medical Center – EMC, Rotterdam, Netherlands): Severe acute respiratory syndrome *coronavirus* Strain HKU39849, Middle East respiratory syndrome *coronavirus*, *Human coronavirus*, Strain NL63 in concentration no more than $1x10^9$ GE/ml and no less than $1x10^7$ GE/ml;
- strains of Smorodintsev Research Institute of Influenza collection: A/Saint Petersburg/NIIG-252/19 (*Influenza virus* A (H3N2)), A/Kaliningrad/75/19 (*Influenza virus* A (H1N1)pdm09), B/Washington/02/19 (*Influenza virus* B Lineage Victoria), B/Yakutsk/NIIG-06/2019 (*Influenza virus* B Lineage Yamagata) in concentration no more than $1x10^9$ GE/ml and no less than $1x10^7$ GE/ml;
- human DNA in concentration of 0.2 mg/ml.

The nonspecific reactions were absent while testing RNA/DNA samples of the above-mentioned microorganisms and human DNA.

The clinical specificity of **AmpliSens® COVID-19-FL** PCR kit was confirmed in laboratory clinical trials.

The information about interfering substances is specified in the *Interfering substances and limitations of using test material samples*.

13.3. Repeatability and reproducibility

Repeatability and reproducibility of SARS-CoV-2 RNA detection were determined by testing of positive and negative model samples. Positive samples were a quality control sample (QCS) containing SARS-CoV-2 RNA with concentration of $5x10^3$ GE/ml, Negative control (C–) was used as a negative sample.

Repeatability conditions included testing in the same laboratory, by the same operator, using the same equipment within a short period of time. Reproducibility conditions included testing different lots of reagent kit in different laboratories, by different operators, on different days, using different equipment. The results are presented in Tables 10, 11.

Table 10

Repeatability

Sample type	Number of repeats	Agreement of results, %	Expected concentration value, lg	Average value of concentration measurement, lg	Standard deviation (SD)	Coefficient of variation (CV), %
Positive	10	100	3.70	3.61	0.13	3.58
Negative	10	100	—	—	—	—

Table 11

Reproducibility

Sample type	Number of repeats	Agreement of results, %	Expected concentration value, lg	Average value of concentration measurement, lg	Standard deviation (SD)	Coefficient of variation (CV), %
Positive	40	100	3.70	3.68	0.13	3.49
Negative	40	100	—	—	—	—

13.4. Trueness (quantitative analysis)

The trueness of quantitative detection of SARS-CoV-2 RNA was determined by testing biological material, in which SARS-CoV-2 RNA was not initially detected, and then a quality control sample containing SARS-CoV-2 RNA in concentrations of $5x10^2$, $7x10^2$, $5x10^3$, $1x10^4$, $1x10^5$, $1x10^7$, $3x10^8$ GE/ml was added.

Table 12

Test material	Number of repeats	Expected concentration value, lg	Average value of concentration measurement, lg	Bias (B), %
Nasopharyngeal and oropharyngeal swabs	12	8.48	8.97	5.79
	12	7.00	7.23	3.28
	12	5.00	5.26	5.18
	12	4.00	4.03	0.75
	10	2.70	2.91	7.69
Sputum / pharyngeal aspirate	12	8.48	8.94	5.50
	12	7.00	7.12	1.77
	12	5.00	5.23	4.55
	12	3.70	3.91	5.69
Bronchoalveolar lavage / bronchial washing fluids	9	8.48	8.88	4.70
	9	7.00	6.97	0.44
	9	5.00	4.99	0.22
	11	3.70	3.78	2.10
	11	2.70	2.93	8.51
Blood plasma	12	8.48	9.07	6.97
	12	7.00	7.11	1.60
	12	5.00	5.18	3.62
	12	4.00	3.99	0.14
Fecal / rectal swab	12	8.48	9.13	7.70
	12	7.00	7.23	3.20
	12	5.00	5.30	6.01
	12	4.00	4.06	1.56
Autopsy material	9	8.48	8.96	5.72
	9	7.00	7.10	1.40
	8	5.00	5.12	2.34
	9	3.70	3.89	5.23

Bias (B) of SARS-CoV-2 RNA concentration logarithm measurement is no more than 20 %.

13.5. Diagnostic characteristics

Table 13

The results of testing AmpliSens® COVID-19-FL PCR kit in comparison with the reference assay

Test material	The results of application of AmpliSens® COVID-19-FL PCR kit		Results of using the reference assay[4]	
			Positive	Negative
Nasopharyngeal and oropharyngeal swabs	160 samples were tested	**Positive**	80	0
		Negative	0	80
Sputum / pharyngeal aspirate	70 samples were tested	**Positive**	35	0
		Negative	0	35
Bronchoalveolar lavage / bronchial washing fluids	70 samples were tested	**Positive**	35	0
		Negative	0	35
Blood plasma	70 samples were tested	**Positive**	35	0
		Negative	0	35
Fecal / rectal swab	70 samples were tested	**Positive**	35	0
		Negative	0	35
Autopsy material	70 samples were tested	**Positive**	35	0
		Negative	0	35
Water sample concentrates, washes from environmental objects	120 samples were tested	**Positive**	20	0
		Negative	0	100

Table 14

Diagnostic characteristics of AmpliSens® COVID-19-FL PCR kit

Test material	Diagnostic sensitivity[5] (with a confidence level of 95 %)	Diagnostic specificity[6] (with a confidence level of 95 %)
Nasopharyngeal and oropharyngeal swabs	100 (95-100) %	100 (95-100) %
Sputum / pharyngeal aspirate	100 (90-100) %	100 (90-100) %
Bronchoalveolar lavage / bronchial washing fluids	100 (90-100) %	100 (90-100) %
Blood plasma	100 (90-100) %	100 (90-100) %
Fecal / rectal swab	100 (90-100) %	100 (90-100) %
Autopsy material	100 (90-100) %	100 (90-100) %
Water sample concentrates, washes from environmental objects	100 (83-100) %	100 (96-100) %

The used test material:

- 227 samples of biological material (nasopharyngeal and oropharyngeal swabs, sputum/pharyngeal aspirate, bronchoalveolar lavage / bronchial washing fluids, blood plasma, fecal/rectal swab, autopsy material) from patients with confirmed clinical diagnosis of COVID-19;
- 28 samples of biological material (nasopharyngeal and oropharyngeal swabs, sputum/pharyngeal aspirate, bronchoalveolar lavage / bronchial washing fluids, blood plasma, fecal/rectal swab, autopsy material) containing SARS-CoV-2 virus at different concentrations;
- 14 environmental samples (water sample concentrates, washes from environmental objects) in which the quantity of SARS-CoV-2 RNA was detected and determined using reference assay;
- 6 environmental samples (water sample concentrates, washes from environmental objects) containing SARS-CoV-2 virus at different concentrations;
- 80 samples of nasopharyngeal and oropharyngeal swabs from patients with different etiology of the disease (*Influenza viruses* A and B, *Human Parainfluenza Virus*, *Human Adenovirus*, *Human Respiratory Syncytial Virus*, *Human Metapneumovirus*, *Human Rhinovirus* and *Human Coronavirus* caused by *HCoV*-NL63, *HCoV*-OC43, *HCoV*-229E, *HCoV*-HKUI);
- 35 samples of sputum / pharyngeal aspirate from patients with different etiology of the disease (*Influenza viruses* A and B, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus pyogenes*, *Staphylococcus aureus*);
- 35 samples of bronchoalveolar lavage / bronchial washing fluids from patients with different etiology of the disease (*Influenza viruses* A and B, *Legionella pneumophila*);
- 35 samples of blood plasma from patients infected with *Human Adenovirus*;
- 35 samples of feces from patients infected with *Rotavirus*;
- 35 samples of autopsy material from patients with different etiology of the disease (*Influenza viruses* A);
- 100 environmental samples (water sample concentrates, washes from environmental objects).

Samples of biological material were obtained from Central Research Institute of Epidemiology as performed by Laboratory of Molecular Methods of Department of Molecular Diagnostics and Epidemiology, Reference Center for Monitoring of Upper and Lower Respiratory Tract Infections by Rospotrebnadzor and Laboratory of Molecular Diagnostics and Epidemiology of Intestinal Infections. Environmental samples were obtained from Reference Center for Monitoring of Acute Intestinal Infections by Rospotrebnadzor.

14. REFERENCES

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2. Viral RNA Load in Mildly Symptomatic and Asymptomatic Children with COVID-19, Seoul, South Korea / M. S. Han, M.-W. Seong, N. Kim et al. // Emerg Infect Dis. – 2020. - 26(10). – P. 2497-2499. doi: 10.3201/eid2610.202449.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® COVID-19-FL** 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
20.01.22 KK	Through the text	The reference numbers of nucleic acid extraction kits and transport mediums were deleted
24.01.23 EM	6. Sampling and handling	The volume of Transport Medium for Storage and Transportation of Respiratory Swabs for nasopharyngeal swabs sampling was changed from1000 to 500 µl

AmpliSens®

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[4] AmpliSens® *Cov*-Bat-FRT PCR kit (REF H-2242-1-CE) in combination with QX100 droplet digital PCR system was used as reference assay.
[5] Relative sensitivity in comparison with applied reference assay.
[6] Relative specificity in comparison with applied reference assay.