AmpliSens® *Norovirus* GI / GII-FRT PCR kit

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

Symbol	Meaning	Symbol	Meaning
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		

1. INTENDED USE

AmpliSens® *Norovirus* GI / GII-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of RNA of *noroviruses* genogroups 1 and 2 *(Norovirus* GI and GII*)* in the biological material (feces) and environmental samples (water sample concentrates) using real-time hybridization-fluorescence detection of amplified products. The material for RT-PCR is RNA samples extracted from test material.

Indications and contra-indications for use of the reagent kit

The reagent kit is used in clinical laboratory diagnostics for the analysis of biological material taken from the persons with suspected *norovirus* infection without distinction of form and presence of manifestation.

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

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)) and simultaneous RNA reverse transcription and amplification of cDNA fragments of the detected viruses 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 TM-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.

AmpliSens® *Norovirus* GI / GII-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 results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE	ROX
cDNA-target	Internal Control-FL (IC) cDNA	*Norovirus* GII cDNA	*Norovirus* GI cDNA
Target gene	Artificially synthesized sequence	gene for capsid protein	gene for capsid protein

3. CONTENT

AmpliSens® *Norovirus* GI / GII-FRT PCR kit is produced in 1 form:
variant FRT-50 F, REF H-2751-1-3-CE.

Variant FRT-50 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-FL *Norovirus* GI / GII	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-buffer-C	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
TM-Revertase (MMlv)	colorless clear liquid	0.015	1 tube
RT-G-mix-2	colorless clear liquid	0.015	1 tube
C+ *Norovirus* GI / GII	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	0.5	1 tube

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

** add **10 µl** of **Internal Control-FL (IC)** during the RNA extraction procedure directly to the sample/lysis mixture (see **RIBO-prep** protocol).

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

4. ADDITIONAL REQUIREMENTS

For sampling and pretreatment

- Sterile 50-60-ml plastic container for sampling, storage and transportation of biological material.
- 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).
- Glycerin for long storage of biological material (feces) in conditions of low-temperature freeze.
- Disposable screwed or tightly closed polypropylene 1.5-ml tubes for sampling and pretreatment.
- Sterile RNase-free pipette tips with aerosol filters (up to 200, 1,000 µl) and without filters (up to 1,000 µl).
- Tube racks.
- Vortex mixer.
- 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 200 µl).
- Tube racks.
- Vortex mixer.
- PCR box.
- Real-time instruments (for example, 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 them briefly.
- Use disposable protective gloves and laboratory coats, 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 samples and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in a 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 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

AmpliSens® *Norovirus* GI / GII-FRT PCR kit is intended for analysis of the RNA extracted with the use of RNA extraction kits from the biological material (feces) and environmental samples (water sample concentrates).

Sampling

Feces are taken from a disposable reservoir (for example, a petrie dish, disposable plastic bag) placed into a bed-pan or disposable diapers (for younger children). When using a disposable diaper for children with liquid stool, a cotton pad should be placed into diaper before the use for obtaining the sufficient quantity of sample.

NOTE: It is forbidden to take feces samples directly from a bed-pan or another reservoir for multiple use (without distinction of disinfection methods).

Using a separate filter tip or disposable spatula transfer about 1.0 g of the sample into special disposable plastic container.

The feces samples can be stored before the pretreatment:

- at the temperature from 18 to 25 °C – for 6 hours,
- at the temperature from 2 to 8 °C – for 3 days.

Only one freeze-thawing cycle is required.

Water sample concentrates are taken 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 1 day,
- at the temperature from minus 24 to minus 16 °C – for 1 month,
- at the temperature not more than minus 68 °C – for a long time,

Only one freeze-thawing cycle is required.

The above mentioned material can be transported at the temperature from 2 to 8 °C for 1 day.

Pretreatment

Pretreatment of *water sample concentrates* is not required.

Feces are to be pretreated.

Fecal suspension preparation:

1. Take the required number of disposable 1.5-ml tubes respectively to the number of samples. Add 1.0 ml of PBS into each tube (use 15-20 % solution of glycerin in PBS when necessary to store the suspension more than 1 day under refrigeration).
2. Using a new one filter tip (or disposable spatula) for each sample add 0.1 g (0.1 ml) of feces into each tube and resuspend thoroughly on vortex due to obtain homogenous suspension. Optimal concentration of suspension is ~ 10 % (by the pellet volume after centrifugation). Sediment the drops from the tube caps by short centrifugation on vortex (no more than 10 sec).

Liquid semitransparent feces are used for express filtration without previous obtaining the suspension.

Express filtration of fecal suspension (for viral and bacterial pathogens detection):

1. For express filtration use two tips up to 1.0 ml (with filter and without filter) and a cut lower part of cotton probe (cotton bud).
2. Put the cut lower part of disposable cotton probe (cotton bud) in the tip without aerosol filter and fix it by pushing into the necked part of the tip.
3. Take 1.0 ml of fecal suspension by the filter tip, put it in the prepared tip with cotton filter and carry out the pressing-filtration into a new disposable tube. In case of difficult filtration it is recommended to decrease the fecal suspension concentration.
4. 100 µl of filtrate is used for RNA extraction.

The pretreated samples of fecal suspensions can be stored before the PCR analysis:

- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature not more than minus 68 °C – for a long time.

Only one freeze-thawing cycle is required.

The pretreated samples of fecal suspension can be transported at the temperature from 2 to 8 °C for 1 day.

Interfering substances and limitations of using test material samples

In order to control the RNA extraction efficiency and amplification reaction 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 (feces) used for the study were selected to assess potential interference.

Model samples of feces without adding and with the addition of potential interfering substances were tested. The concentration of each potential interfering substance is specified in Table 2. Model samples of feces contained quality control samples (QCSs) in concentrations corresponding to the limit of detection.

Table 2

Type of potential interferent	Potential interferent	Tested concentration in a sample	Interference presence
Endogenous substances	Whole blood	40 %	Not detected
	Fecal fats	40 %	Not detected
	Mucin (mucus)	3 % (the preparation weight to material volume)	Not detected
Exogenous substances	'Enterofuril' oral suspension	4.25 mg/ml	Not detected
	'Enterosgel', oral paste (sweet)	174.75 mg/ml	Not detected
	Dextrin (Russia)	68.6 mg/ml	Not detected

7. WORKING CONDITIONS

AmpliSens® *Norovirus* GI / GII-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

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

- **RIBO-prep**.

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

NOTE:
The volumes of reagents and samples when the RNA is extracted by the RIBO-prep reagent kit:

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

Add **10 µl** of **Internal Control-FL (IC)** to each tube.

The volume of the test sample is **100 µl.**

Add **100 µl** of **Negative Control (C–)** reagent into the tube labeled C– (Negative Control of Extraction).

The volume of elution is **50-100 µl** depending on number of PCR kits which will be used for analysis of obtained RNA.

NOTE: It is recommended to carry out the RT-PCR just after the 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 not more than minus 68 °C for 1 year. Only one freeze-thawing cycle is required.

8.2. Preparing reverse transcription and PCR

8.3.1 Preparing tubes for 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 *Norovirus* GI / GII,**
   - **5 µl** of **PCR-buffer-C,**
   - **0.5 µl** of **Polymerase (TaqF),**
   - **0.25 µl** of **TM-Revertase (MMlv),**
   - **0.25 µl** of **RT-G-mix-2**.

Prepare the reaction mixture for the total number of test and control samples plus one 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		10.00	5.00	0.25	0.50	0.25
Number of test samples	Number of reactions [1]	PCR-mix-FL	PCR-buffer-C	RT-G-mix-2	Polymerase (TaqF)	TM-Revertase (MMlv)
2	6	60	30	1.5	3.0	1.5
4	8	80	40	2.0	4.0	2.0
6	10	100	50	2.5	5.0	2.5
8	12	120	60	3.0	6.0	3.0
10	14	140	70	3.5	7.0	3.5
12	16	160	80	4.0	8.0	4.0
14	18	180	90	4.5	9.0	4.5
16	20	200	100	5.0	10.0	5.0
18	22	220	110	5.5	11.0	5.5
20	24	240	120	6.0	12.0	6.0
22	26	260	130	6.5	13.0	6.5
24	28	280	140	7.0	14.0	7.0
26	30	300	150	7.5	15.0	7.5
28	32	320	160	8.0	16.0	8.0

NOTE: Prepare the reaction mixture just before use.

2. Thaw the tube with **PCR-mix-FL *Norovirus* GI / GII.** 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 *Norovirus* GI / GII, PCR-buffer-C, Polymerase (TaqF), TM-Revertase (MMlv)** 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.

[1] Number of reactions = number of test samples + controls of extraction (C–) and RT-PCR (C+, NCA) + one extra reaction.

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5. Transfer **15 µl** of the prepared reaction mixture to each tube. Discard the unused reaction mixture.
6. Add **10 µl** of **RNA samples** obtained at the RNA extraction stage.

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

7. Carry out the control reactions:
   - **C+** – Add **10 µl** of **C+ *Norovirus* GI / GII** to the tube labeled **C+** (Positive Control of Amplification).
   - **NCA** – Add **10 µl** of **TE-buffer** to the tube labeled **NCA** (Negative 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).

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

NOTE: Carry out the RT-PCR just after the mix of reaction mixture and RNA-samples and controls. The time of adding the samples in the reaction mixture and reaction run should not exceed 10-15 min.

8.3.2. Amplification

1. Create a temperature profile on your instrument as follows[2]:

Table 4

AmpliSens unified amplification program for rotor-type[3] and plate-type[4] instruments

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	**50**	**15 min**	**–**	**1**
2	**95**	**15 min**	**–**	**1**
3	**95**	**10 s**	**–**	**45**
	60	**20 s**	**FAM, JOE, ROX**	

NOTE: Any combination of the tests (including tests with reverse transcription and amplification) can be performed in one instrument simultaneously with the use of the unified amplification program. If several tests in "multiprime" format are carried out simultaneously, the detection is enabled in other used channels except for the specified ones.

Table 5

Amplification program for rotor-type[3] and plate-type[4] instruments

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	**50**	**30 min**	**–**	**1**
2	**95**	**15 min**	**–**	**1**
3	**95**	**10 s**	**–**	**45**
	60	**25 s**	**FAM, JOE, ROX**	
	72	**10 s**	**–**	

NOTE: The given program (table 5) can be used for all AmpliSens® PCR kits, intended for detection and differentiation of DNA/RNA of microorganisms inducing acute intestinal infections, with a possibility of its simultaneous use in one run. If other tests are carried out simultaneously, the detection is enabled in other used channels.

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 used by measuring fluorescence signal accumulation in three channels:

Table 6

Channel for the fluorophore	FAM	JOE	ROX
Amplification product	Internal Control-FL (IC) cDNA	*Norovirus* GII cDNA	*Norovirus* GI cDNA

Results are interpreted by the crossing (or not-crossing) the S-shaped (sigmoid) 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.

Principle of interpretation is the following:

Table 7

Results interpretation

***Ct* value in the channel for the fluorophore**			**Result**
FAM	**JOE**	**ROX**	
< boundary value	absent or > boundary value	absent or > boundary value	*Norovirus* GI, GII RNA **is not detected**
determined or absent	< boundary value	absent or > boundary value	*Norovirus* GII RNA **is detected**
determined or absent	absent or > boundary value	< boundary value	*Norovirus* GI RNA **is detected**
determined or absent	< boundary value	< boundary value	*Norovirus* GI, GII RNA **is detected**
absent or > boundary value	absent or > boundary value	absent or > boundary value	**Invalid** result*

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

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

The results for controls of extraction and amplification stages must meet the criteria given in Table 8 and in the *Important Product Information Bulletin* enclosed to the PCR kit.

Table 8

Results for controls

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

Interpretation of some test samples is not possible if the results for the controls deviate from the results specified above (see *10.Troubleshooting*).

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 specified channels for fluorophores (see table 8) is greater than the boundary value or absent. The results interpretation is not possible for samples in which the analyzed target RNA was not detected, the PCR analysis should be repeated for these samples. For the samples in which the analyzed microorganism RNA was detected, it is necessary to follow the steps specified in point 4.
2. For the Negative Control of Extraction (C–):
   a) The *Ct* value determined in the channels for the JOE and/or ROX fluorophores is less than the boundary value. The contamination of laboratory with amplification products or cross-contamination of reagents / test samples is probable at any stage of PCR analysis. The results interpretation is not possible for the samples in which the analyzed target RNA was detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the RNA extraction stage) should be repeated for these samples;
   b) The *Ct* value determined in the channel for the FAM fluorophore is absent or greater than the boundary value. The results interpretation for the test samples is carried out according to Table 7.
3. For the Negative Control of reverse transcription and amplification (NCA):
   a) The *Ct* value determined in the channels for the JOE and/or ROX fluorophores is less than the boundary value. The contamination of laboratory with amplification products or cross-contamination of reagents / test samples is probable at any stage of PCR analysis. The results interpretation is not possible for the samples in which the analyzed target RNA was detected. Measures for detecting and elimination of contamination source must be taken. The amplification should be repeated for these samples;
   b) The *Ct* value determined in the channel for the FAM fluorophore is less than the boundary value. The contamination of laboratory with amplification products or cross-contamination of reagents / test samples is probable at any stage of PCR analysis. The results interpretation is not possible for the samples in which the analyzed target RNA was not detected. Measures for detecting and elimination of contamination source must be taken. The amplification should be repeated for these samples.
4. The *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence (in the "raw" data view mode) is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level. If the result has been obtained with the correct level of threshold line (base line), the amplification 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® *Norovirus* GI / GII-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days. PCR kit can be transported at 2–25 °C for no longer than 3 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Norovirus* GI / GII-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-FL *Norovirus* GI / GII, PCR-buffer-C, polymerase (TaqF), TM-Revertase (MMlv) and RT-G-mix-2). All components of the **AmpliSens® *Norovirus* GI / GII-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 *Norovirus* GI / GII, PCR-buffer-C, polymerase (TaqF), TM-Revertase (MMlv) and RT-G-mix-2 are to be stored at the temperature from minus 24 to minus 16 °C

NOTE: PCR-mix-FL *Norovirus* GI / GII is to be kept away from light

13. SPECIFICATIONS

13.1. Analytical sensitivity (limit of detection)

Table 9

Microorganism	Test material	Nucleic acid extraction kit	PCR kit	Analytical sensitivity (limit of detection), GE/ml[5]
Norovirus GI	water sample concentrates	RIBO-prep	variant FRT-50 F	$1x10^3$
	feces	RIBO-prep	variant FRT-50 F	$5x10^3$
Norovirus GII	water sample concentrates	RIBO-prep	variant FRT-50 F	$1x10^3$
	feces	RIBO-prep	variant FRT-50 F	$5x10^3$

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

[2] The amplification programs (tables 4, 5) are equivalent for the use with this PCR kit.
[3] For example, Rotor-Gene Q (QIAGEN, Germany).
[4] For example, CFX 96 (Bio-Rad, USA).

[5] Number of genome equivalents (GE) of the microorganism per 1 ml of the test material sample;

13.2. Analytical specificity

The analytical specificity of **AmpliSens® *Norovirus* GI / GII-FRT** 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 PCR kit specificity was tested on the following panels of microorganisms' strains and RNA/DNA samples:

1. *Noroviruses* genogroups 1 and 2 (*Norovirus* GI – 37 samples, *Norovirus* GII – 100 samples) (the clinical isolates; the specificity was proved by the direct sequencing of the nucleotide sequences);
2. Human enteroviruses (the representatives of different genetic clusters – *Human echovirus* 2, 6, 9, 11, 14, 15, 16, 17, 18, 30; *Human coxsackievirus* A4, A5, A6, A9, A16, B4, B5, *Human poliovirus* 1, 2, 3 (Sabin1, Sabin2, Sabin3)); *Influenza viruses* A (H13N2, H9N2, H8N4, H2N3, H4N6, H11N6, H12N5, H3N8, H1N1, H6N2, H10N7, H5N1), B, *Rhinoviruses*, *RS viruses*, *Human adenoviruses* – 3, 5, 7, 37, 40, 41 types (the clinical isolates; the species affiliation was proved by the direct sequencing of the nucleotide sequences);
3. Human DNA, strains of *Acinetobacter baumanii* ATCC® 19606™, *Bacteroides fragilis* ATCC® 25285™, *Bordetella bronchiseptica* ATCC® 10580™, *Bordetella bronchiseptica* ATCC® 4617™, *Bordetella pertussis* ATCC® 9340™, *Candida albicans* ATCC® 14053™, *Candida guilliermondii* ATCC® 6260™, *Candida krusei* ATCC® 14243™, *Clostridium difficile* ATCC® 9689™, *Clostridium septicum* ATCC® 12464™, *Corynebacterium jeikeium* ATCC® 43734™, *Corynebacterium xerosis* ATCC® 373™, *Eggerthella lenta (Eubacterium lentum)* ATCC® 43055™, *Enterobacter aerogenes* ATCC® 13048™, *Enterobacter cloacae* ATCC® 13047™, *Enterococcus faecalis* ATCC® 29212™, *Enterococcus faecalis (vancomycin resistant)* ATCC® 51299™, *Enterococcus faecium* ATCC® 35667™, *Erysipelothrix rhusiopathiae* ATCC® 19414™, *Escherichia coli* ATCC® 25922™, *Escherichia coli* ATCC® 35218™, *Gardnerella vaginalis* ATCC® 14018™, *Haemophilus infuenzae* ATCC® 33930™, *Haemophilus influenzae* ATCC® 9006™, *Haemophilus influenzae* ATCC® 10211™, *Haemophilus parainfluenzae* ATCC® 7901™, *Klebsiella oxytoca* ATCC® 49131™, *Klebsiella pneumoniae* ATCC® 27736™, *Listeria grayi (murrayi)* ATCC® 25401™, *Listeria innocua* ATCC® 33090™, *Listeria monocytogenes* ATCC® 7644™, *Moraxella (Branhamella) catarrhalis* ATCC® 25238™, *Moraxella (Branhamella) catarrhalis* ATCC® 8176™, *Neisseria meningitidis* ATCC® 13102™, *Neisseria meningitidis* ATCC® 13090™, *Neisseria lactamica* ATCC® 23970™, *Neisseria gonorrhoeae* ATCC® 19424™, *Neisseria gonorrhoeae* ATCC® 49926™, *Proteus mirabilis* ATCC® 12453™, *Proteus vulgaris* ATCC® 6380™, *Propionibacterium acnes* ATCC® 11827™, *Pseudomonas aeruginosa* ATCC® 15442™, *Rhodococcus equi* ATCC® 6939™, *Salmonella enterica subsp. enterica serovar Typhimurium* ATCC® 14028™, *Serratia marcescens* ATCC® 14756™, *Staphylococcus aureus* (MRSA) ATCC® 43300™, *Staphylococcus aureus* ATCC® 29213™, *Staphylococcus aureus* ATCC® 25923™, *Staphylococcus aureus* ATCC® 33862™, *Staphylococcus aureus* (MRSA) ATCC® 33591™, *Staphylococcus aureus subsp. aureus* ATCC® 12600™, *Staphylococcus epidermidis* ATCC® 12228™, *Staphylococcus haemolyticus* ATCC® 29970™, *Staphylococcus saprophyticus* ATCC® 49907™, *Stenotrophomonas maltophilia* ATCC® 13637™, *Streptococcus agalactiae* ATCC® 12386™, *Streptococcus agalactiae* ATCC® 13813™, *Streptococcus equisimilis* ATCC® 12388™, *Streptococcus equi subsp. equi* ATCC® 9528™, *Streptococcus bovis* (Group D) ATCC® 9809™, *Streptococcus mutans* ATCC® 35668™, *Streptococcus pneumoniae* ATCC® 49619™, *Streptococcus pneumoniae* ATCC® 6303™, *Streptococcus pneumoniae* ATCC® 27336™, *Streptococcus pneumoniae* ATCC® 6305™, *Streptococcus pyogenes* ATCC® 19615™, *Streptococcus salivarius* ATCC® 13419™, *Streptococcus uberis* ATCC® 700407™, *Vibrio parahaemolyticus* ATCC® 17802™, *Vibrio vulnificus* ATCC® 27562™, *Moraxella catarrhalis* ATCC® 25240™, *Corynebacterium minutissimum* ATCC® 23348™.

The nonspecific reactions were absent while testing 2nd and 3rd panels as well as human DNA.

The clinical specificity of **AmpliSens® *Norovirus* GI / GII-FRT** 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 were determined by testing positive and negative model samples. Positive samples were quality control samples (QCS) containing *Norovirus* GI RNA, *Norovirus* GII RNA with concentration of $1x10^3$ 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 PCR kit in different laboratories, by different operators, on different days, using different equipment. The results are presented in Table 10.

Table 10

Sample type	Repeatability		Reproducibility	
	Number of samples	**Agreement of results, %**	**Number of samples**	**Agreement of results, %**
Norovirus GI	10	100	40	100
Norovirus GII	10	100	40	100
Negative	10	100	40	100

13.3. Diagnostic characteristics

For diagnostic characteristics evaluation were used:

- 609 samples of feces obtained from group of patients with diarrhea caused by 1 and 2 genotypes of norovirus infection. The diagnosis of norovirus infection was proved by immunoenzymatic method (reagent kit RIDASCREEN® Norovirus 3rd Generation for detection of norovirus produced by R-Biopharm AG, Germany).
- 211 samples of feces obtained from group of patients with diarrhea caused by enteric infections with another etiology. The diagnosis of norovirus infection was not proved by immunoenzymatic method (reagent kit RIDASCREEN® Norovirus 3rd Generation for detection of norovirus produced by R-Biopharm AG, Germany).
- 200 model samples of water contaminated with quality control sample (QCS) containing *Norovirus* GI RNA. *Norovirus* GI RNA concentration in model samples was $1x10^3$ copies/ml;
- 200 model samples of water contaminated with quality control sample (QCS) containing *Norovirus* GII RNA. *Norovirus* GII RNA concentration in model samples was $1x10^3$ copies/ml;

The Sanger's method of direct sequencing of amplified products was used as a reference method for *Norovirus* GI detection. **AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT** PCR kit was used as the kit for comparison for *Norovirus* GII detection.

Table 11

Diagnostic characteristics of AmpliSens® *Norovirus* GI / GII-FRT PCR kit

Detected pathogen	Samples type	Diagnostic sensitivity (with a confidence level of 95 %), in the interval%	Diagnostic specificity (with a confidence level of 95 %), in the interval%
Norovirus GI	feces	98.6-100	98.6-100
	water samples	98.5-100	98.5-100
Norovirus GII	feces	99.3-100	98.6-100
	water samples	98.5-100	98.5-100

14. REFERENCES

1. Atmar RL, Estes MK. The epidemiologic and clinical importance of norovirus infection. Gastroenterol Clin North Am. 2006;35(2):275-290.

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® *Norovirus* GI / GII-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
15.08.18 PM	9. Data analysis	Results interpretation was specified
	13. Specification	The information about diagnostic characteristics evaluation was changed
03.10.18 EM	6. Sampling and handling	The information about the amplification reaction inhibition was deleted
10.09.19 EM	Through the text	The text formatting was changed. Corrections according to the template
	14. References	The section was actualized
02.06.20 MM	Footer	The phrase "Not for use in the Russian Federation" was added
11.03.21 MM	—	The name, address and contact information for Authorized representative in the European Community was changed
20.10.21 MM	Through the text	The **RIBO-prep** REF K2-9-Et-50-CE was changed to **RIBO-prep** REF K2-9-Et-100-CE
21.01.22 MM	Through the text	The reference numbers of nucleic acid extraction kit was deleted
01.11.22 EM	Through the text	All the sections were updated according to the template
	1. Intended use	The information about the test material for RT-PCR was added; the subsection "Indications and contra-indications for use of the reagent kit" was added
	6. Sampling and handling	The subsection "Potential interfering substances" was added
	9. Data analysis	The information in the table with results interpretation for test samples was updated
	10. Troubleshooting	The information of the section was totally updated in accordance with the Russian Instruction
	13. Specifications	The reference to the section to the subsection "Interfering substances and limitations of using test material samples" was added; the section "Repeatability and reproducibility" was added; the values of diagnostic sensitivity and diagnostic specificity were changed in accordance with the Russian Instruction

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