

# AmpliSens® Norovirus GI / GII-FRT PCR kit



For Professional Use Only

## Instruction Manual

### KEY TO SYMBOLS USED

	Catalogue number		Contains sufficient for <n> tests
	Batch code		Use-by-date
	Research Use Only		Consult instructions for use
	Version		Keep away from sunlight
	Temperature limit		Negative control of amplification
	Manufacturer		Negative control of extraction
	Date of manufacture		Positive control of amplification
	Caution		Internal control

### 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 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:** For research use only. Not for diagnostic procedures

### 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, 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 (MMIv)	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 distinctly 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 (QCS) in concentrations corresponding to the limit of detection.

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.

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.

**NOTE:**

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.

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.

**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 (MMIv),**
- **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

Reagent volume per one reaction, µl		Reagent volume for specified number of reactions				
Number of test samples	Number of reactions <sup>1</sup>	10.00	5.00	0.25	0.50	0.25
		PCR-mix-FL	PCR-buffer-C	RT-G-mix-2	Polymerase (TaqF)	TM-Revertase (MMIv)
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 (MMIv)** 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.

<sup>1</sup> Number of reactions = number of test samples + controls of extraction (C-) and RT-PCR (C+, NCA) + one extra reaction.

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<sup>2</sup>:

Table 4

AmpliSens unified amplification program for rotor-type <sup>3</sup> and plate-type <sup>4</sup> 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	

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 "multiplex" format are carried out simultaneously, the detection is enabled in other used channels except for the specified ones.

**NOTE:**

Table 5

Amplification program for rotor-type <sup>3</sup> and plate-type <sup>4</sup> 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	–	

The given program (table 5) can be used for all AmpliSens<sup>®</sup> 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.

**NOTE:**

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin*.

3. Insert tubes into the reaction module of the device.

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

**NOTE:**

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

\* 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.

<sup>2</sup> The amplification programs (tables 4, 5) are equivalent for the use with this PCR kit.

<sup>3</sup> For example, Rotor-Gene Q (Qiagen, Germany).

<sup>4</sup> For example, CFX 96 (Bio-Rad, USA).

## Results for controls

Table 8

Control	Stage for control	<i>Ct</i> 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:

- 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.
- For the Negative Control of Extraction (C-):
  - 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;
  - 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.
- For the Negative Control of reverse transcription and amplification (NCA):
  - 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;
  - 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.
- 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.

## 11. TRANSPORTATION

AmpliSens<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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 <sup>5</sup>
Norovirus GI	water sample concentrates	RIBO-prep	variant FRT-50 F	1x10 <sup>3</sup>
	feces	RIBO-prep	variant FRT-50 F	5x10 <sup>3</sup>
Norovirus GII	water sample concentrates	RIBO-prep	variant FRT-50 F	1x10 <sup>3</sup>
	feces	RIBO-prep	variant FRT-50 F	5x10 <sup>3</sup>

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® 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 baumannii* 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 influenzae* 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 2<sup>nd</sup> and 3<sup>rd</sup> panels as well as human DNA.

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<sup>5</sup> 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

### 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.

