CE

IVD

For Professional Use Only

AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

Federal Budget Institute of Science “Central Research Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

TABLE OF CONTENTS

1.INTENDED USE

AmpliSens® *Rotavirus* / *Norovirus* / *Astrovirus*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of *rotavirus* A, *norovirus* genotype 2, and *astrovirus* RNA in the environmental samples (water sample concentrates) and clinical material (feces) using real-time hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

Detection of *rotavirus* A, *norovirus* genotype 2, and *astrovirus* RNA includes RNA extraction from test samples and reverse transcription of RNA into cDNA combined with real-time PCR amplification of cDNA (RT-PCR). 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® *Rotavirus* / *Norovirus* / *Astrovirus*-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-87-rec (IC)). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

AmpliSens® *Rotavirus* / *Norovirus* / *Astrovirus*-FRT PCR kit uses “hot-start,” which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *Rotavirus* / *Norovirus* / *Astrovirus*-FRT PCR kit is produced in 1 form:

AmpliSens® *Rotavirus* / *Norovirus* / *Astrovirus*-FRT PCR kit variant FRT-50 F, **REF** R-V40(RG,iQ)-CE.

AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT PCR kit variant FRT-50 F includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
RT-PCR-mix-1-FEP/FRT *Rotavirus / Astrovirus*	clear liquid from colorless to light lilac colour	0.6	1 tube
RT-PCR-mix-1-FEP/FRT *Norovirus* / STI	clear liquid from colorless to light lilac colour	0.6	1 tube
RT-PCR-mix-2-FEP/FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
TM-Revertase (MMlv)	colorless clear liquid	0.015	2 tubes
RT-G-mix-2	colorless clear liquid	0.015	2 tubes
Positive Control cDNA *Rotavirus*-Flu / *Astrovirus* (C+ *Rotavirus / Astrovirus*)	colorless clear liquid	0.1	1 tube
Positive Control cDNA *Norovirus* genotype 2-Flu /STI (C+ *Norovirus* genotype 2 / STI)	colorless clear liquid	0.1	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)*	colorless clear liquid	1.6	1 tube
Internal Control STI-87-rec (IC)**	colorless clear liquid	0.12	5 tubes
RNA-eluent***	colorless clear liquid	1.2	5 tubes

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

** add **10 µl** of **Internal Control STI-87-rec (IC)** during the RNA extraction procedure directly to the sample/lysis mixture (see the RIBO-sorb **REF** K2-1-Et-50-CE, or RIBO-prep **REF** K2-9-Et-50-CE protocols).

***must be used in the extraction procedure.

AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT PCR kit is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 100 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.

- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany), iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA)).
- Disposable polypropylene PCR tubes:
  a) 0.2-ml tube (flat cap, nonstriped) for 36-well rotor if a rotor-type instrument is used.
  b) 0.2-ml tube (domed cap) if plate-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers 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 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 samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- 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

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

AmpliSens® *Rotavirus* / *Norovirus* / *Astrovirus*-FRT PCR kit is intended for analysis of the RNA extracted with RNA extraction kits from:

- water sample concentrates (pretreatment is not required),
- feces (pretreatment should be carried out as described in manufacturer's handbook [1]).

7. WORKING CONDITIONS

AmpliSens® *Rotavirus* / *Norovirus* / *Astrovirus*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA extraction

It is recommended that the following nucleic acid extraction kits are used:

- RIBO-prep **REF** K2-9-Et-50-CE;
- RIBO-sorb **REF** K2-1-Et-50-CE;

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

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

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

Extract RNA according to the manufacturer's protocols.

In case of extracting with RIBO-sorb reagent kit, the volume of **Internal Control STI-87-rec (IC)** reagent added to each tube is **10 µl**.

Use RNA-eluent included in this PCR kit during RNA extraction.

8.2. Preparing RT-PCR

8.2.1. Preparing tubes for RT-PCR

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

The type of tubes depends on the type of PCR real-time instrument.

Use disposable filter tips for adding reagents, cDNA and control samples into tubes.

1. Reaction mixture components should be mixed just before analysis with calculating for the required reaction number (including test and control samples) according to Table 1. Note that even for analysis of one test or control RNA sample it is necessary to carry out all controls of RT-PCR (positive (C+) and negative (NCA)) for each RT-PCR-mix-1. It is recommended to mix the reagents for an even reaction number to ensure more exact dosage.
2. Take the required number of tubes for amplification of test and control samples.
3. To prepare the reaction mixture, mix one of the **RT-PCR-mix-1-FEP/FRT (RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus*** or **RT-PCR-mix-1-FEP/FRT *Norovirus* / STI), RT-PCR-mix-2 FEP/FRT, polymerase (TaqF),** and **TM-Revertase (MMlv)** according to Table 1. Vortex the tubes thoroughly. Make sure that there are no drops on the walls of the tubes.
4. Transfer **15 µl** of the prepared mixture to the prepared tubes. Dispose of the unused reaction mixture.

Table 1

Scheme of reaction mixture preparation

		Reagent volume for specified number of reactions, µl				
Reagent volume per one reaction, µl		**10.00**	**5.00**	**0.25**	**0.50**	**0.25**
Number of test samples	**Number of reactions [1]**	**RT-PCR-mix-1-FEP/FRT**	**RT-PCR-mix-2-FEP/FRT**	**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**

[1] Test samples (N) + control of RNA extraction + 2 controls of RT-PCR + extra reaction (N+1+2+1).

5. Add **10 µl** of **RNA** obtained at the RNA extraction stage to the prepared tubes using tips with aerosol filter.

Avoid transferring sorbent beads together with the RNA sample in case of extraction with the RIBO-sorb kit.

6. Carry out the control amplification reactions:

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

C+*Rotavirus / Astrovirus* – Add **10 µl** of **Positive Control cDNA *Rotavirus*-Flu / *Astrovirus* (**C+***Rotavirus / Astrovirus***)** (in case of using RT-PCR-mix-1-FEP/FRT *Rotavirus / Astrovirus)* to the tube labeled C+***Rotavirus / Astrovirus*** (Positive Control of Amplification)

C+*Norovirus* genotype 2 / STI – Add **10 µl** of **Positive Control cDNA *Norovirus* genotype 2-Flu / STI (**C+***Norovirus* **genotype 2 / STI)** (in case of using RT-PCR-mix-1-FEP/FRT *Norovirus* / STI) to the tube labeled C+***Norovirus* genotype 2 / STI** (Positive Control of Amplification)

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

8.2.2. Amplification

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

Table 2

Amplification program

	Rotor-type Instruments[2]			Plate-type Instruments[3]		
Step	***Temperature, °C***	***Time***	**Repeats**	***Temperature, °C***	***Time***	**Repeats**
1	**50**	30 min	1	**50**	30 min	1
2	**95**	15 min	1	**95**	15 min	1
3	**95**	10 s	45	**95**	10 s	45
	60	25 s *fluorescent signal detection*		**60**	25 s *fluorescent signal detection*	
	72	10 s		**72**	10 s	

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores (other channels are enabled if several tests are simultaneously carried out in a single run).

2. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin* and Guidelines [2].
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

[2] Rotor-Gene 3000, Rotor-Gene 6000, or equivalent.
[3] iCycler iQ5, Mx3000P, or equivalent.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels (see table 3).

Table 3

Correspondence table of detection channels, RT-PCR-mixes-1 and pathogens

Channel for fluorophore	RT-PCR-mix-1-FEP/FRT ***Rotavirus* / *Astrovirus***	RT-PCR-mix-1-FEP/FRT ***Norovirus* / STI**
FAM	*Rotavirus* grA cDNA	Internal Control STI-87-rec
JOE	*Astrovirus* cDNA	*Norovirus* G2 cDNA

Result interpretation

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.

Results should be interpreted in accordance with Table 4 and *Important Product Information Bulletin*.

Table 4

Interpretation of results

Channel for fluorophore	RT-PCR-mix-1-FEP/FRT ***Rotavirus* / *Astrovirus***	RT-PCR-mix-1-FEP/FRT ***Norovirus* / STI**
FAM	< boundary value *Rotavirus* grA RNA is detected	< boundary value IC cDNA is detected. The result of sample is valid
	Absent or > boundary value *Rotavirus* grA RNA is not detected[4]	Absent or > boundary value Invalid result[5]
JOE	< boundary value *Astrovirus* RNA is detected	< boundary value *Norovirus* G2 RNA is detected
	Absent or > boundary value *Astrovirus* RNA is not detected[4]	Absent or > boundary value *Norovirus* G2 RNA is not detected[4]

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

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 5).

[4] Only if the *Ct* value for RT-PCR-mix-FEP/FRT *Norovirus* / STI in the FAM channel is less than the boundary value.

[5] If *Ct* value for RT-PCR-mix-FEP/FRT *Norovirus* / STI in the FAM channel is absent or greater than the boundary value, the negative result obtained with the other PCR-mix-1 is considered invalid; therefore, the sample should be examined once again starting from RNA extraction.

Table 5

Results for controls

RT-PCR-mix-1	Control	Stage for control	*Ct* value in the channel for fluorophore	
			FAM	JOE
RT-PCR-mix-1-FEP/FRT *Norovirus* / STI	**C–**	RNA extraction	≤ boundary value	Absent or > boundary value
	NCA	PCR	Absent or > boundary value	Absent or > boundary value
	C+*Norovirus* genotype 2 / STI	PCR	< boundary value	< boundary value
RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus*	**C–**	RNA extraction	Absent or > boundary value	Absent or > boundary value
	NCA	PCR	Absent or > boundary value	Absent or > boundary value
	C+*Rotavirus / Astrovirus*	PCR	< boundary value	< boundary value

10. TROUBLESHOOTING

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

1. If the *Ct* value determined for the Positive Control of amplification (C+) in the channels for the JOE and FAM fluorophores is greater than the boundary *Ct* value, the amplification and detection should be repeated for all samples in which the signal in the channels for the JOE and FAM fluorophores was greater than the boundary value with the appropriate RT-PCR-mix.
2. If the signal for the Negative control of extraction (C–) (except for RT-PCR-mix-1-FEP/FRT *Norovirus* / STI) and/or the Negative control of amplification (NCA) in the channels for the JOE and FAM fluorophores is less than the boundary value, PCR should be repeated (starting from RNA extraction stage) for all samples in which the pathogen cDNA was detected.

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

11. TRANSPORTATION

AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for RT-PCR-mix-1-FEP/FRT *Rotavirus* /

Astrovirus, RT-PCR-mix-1-FEP/FRT *Norovirus* / STI, RT-PCR-mix-2-FEP/FRT, polymerase (TaqF), TM-Revertase (MMlv), and RT-G-mix-2). All components of the **AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT** PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus*, RT-PCR-mix-1-FEP/FRT *Norovirus* / STI, RT-PCR-mix-2-FEP/FRT, polymerase (TaqF), TM-Revertase (MMlv), and RT-G-mix-2 are to be stored at temperature from minus 24 to minus 16 °C.

RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus* and RT-PCR-mix-1-FEP/FRT *Norovirus* / STI are to be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT** PCR kit is specified in the table below.

Pathogen	Test material	RNA/DNA extraction kit	PCR kit	Analytical sensitivity, GE/ml[6]
Rotavirus A	Feces	RIBO-prep	PCR kit variant FRT-50 F	1×10^4
Norovirus genotype 2	Feces	RIBO-prep	PCR kit variant FRT-50 F	5×10^3
Astrovirus	Feces	RIBO-prep	PCR kit variant FRT-50 F	1×10^4

13.2. Specificity

The analytical specificity of **AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT** PCR kit is ensured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

Specificity was confirmed on the following microorganism strains: *Enterovirus* strains (Coxsakie B1, B2, B3, B4, B5, and B6; Polio (Sabin) I, II, and III); *Adenovirus* serogroups 5 and 7; *influenza virus* A (H13N2, H9N2, H8N4, H2N3, H4N6, H11N6, H12N5, H3N8, H1N1, H6N2, H10N7, and H5N1) and B; *rhinoviruses*; *RS viruses*; human *adenovirus* types 3, 5, 7, 37, and 40; *Salmonella enteritidis* S-6, *S.choleraesuis* 370, *S.typhimurium* 371, *S.dublin* 373, *S.typhi* C1, *S.abortusovis 372*, and *S.gallinarum-pullorum;*, *Shigella flexneri* 851b; *Campylobacter fetus* ssp. *fetus* 25936 and *C.jejuni* ssp. *jejuni* 43435; *Clebsiella* K 65 SW4; *Listeria monocitogenes* USKHCH 19 and *L.monocitogenes* USKHCH 52; *Proteus vulgaris* 115/98; *Pseudomonas aeruginosa* DN c1; *Staphilococcus*

[6] Genome equivalents (GE) of the microorganism per 1 ml of a sample.

aureus 653 and *S. aureus* 29112; *Morganella morganii* 619 c 01; and *Enterobacter faecalis* 356; as well as 44 *Norovirus* isolates of different gene clusters of genotypes 1 and 2; 40 *Rotavirus* strains of different [P]G types, 19 *Astrovirus* strains of serogroups 1, 2, 4, 5, and 8; and 15 *Adenovirus* strains of different types and the following bacterial strains (see table 6).

Table 6

The panel of bacterial pathogens
Center for Disease Control and Prevention (CDC, USA)

Strain ID	Organism	Strain ID	Organism
K2033	*Salmonella* ser. Grumpensis	K2015	*Salmonella* ser. Oranienburg
K1806	*Salmonella* ser. Newport	AM01144	*Salmonella* ser. Newport
K2077	*Salmonella* ser. Enteriditis	K1810	*Salmonella* ser. Anatum
83-99	*Salmonella* ser. Typhimurium	K1991	*Salmonella* ser. Typhimurium
PS505	*Shigella boydii*	K1898	*Salmonella* ser. Heidelberg
PS408	*Shigella sonnei*	PS555	*Shigella boydii*
B4003	*Shigella sonnei*	F6446	*Shigella dysenteriae*
PS801	*Shigella dysenteriae*	S821X1	*Shigella dysenteriae* type 1
C898	*Shigella dysenteriae* type1	S177X1	*Shigella dysenteriae* type 1
F2035	*Shigella flexneri*	S3314	*Shigella dysenteriae* type 2
E2539-C1	Enterotoxigenic *Escherichia coli* (ETEC)	PS071	*Shigella flexneri*
H10407	Enterotoxigenic *Escherichia coli* (ETEC)	PS050	*Shigella flexneri*
F1008	Enterotoxigenic *Escherichia coli* (ETEC)	F7862	*Shigella flexneri*
EDL 933	Shiga-toxin *E. coli* (STEC)	TX1	Enterotoxigenic *Escherichia coli* (ETEC)
3543-01	Shiga-toxin *E. coli* (STEC)	3525-01	Shiga-toxin *Escherichia coli* (STEC)
4752-71	*Proteus vulgaris*	25922	*Escherichia coli* O6:H1
QA/QC	*Citrobacter freundii*	621-64	*Citrobacter freundii*
QA/QC	*Aeromonas*	3910-68	*Aeromonas spp.*
3043-74	*Serratia marcescens*	E9113	*Vibrio cholerae*
QA/QC	*Serratia marcescens*	501-83	*Edwardsiella spp.*
F7894	*Vibrio vulnificus*	587-82	*Providencia stuartii*
F8515	*Yersinia enterocolitica*	27853	*Pseudomonas aeruginosa*
F8510	*Yersinia enterocolitica*	D4989	*Helicobacter cineadi*
K4299	*Vibrio parahaemolyticus*	D6827	*Helicobacter pullorum*
F9835	*Vibrio parahaemolyticus*	D5127	*Helicobacter pylori*
K2023	*Salmonella* ser. Kentucky	D2686	*Arcobacter butzleri*
K1684	*Salmonella* O-1, 4, 12 gr. B		

There were no nonspecific test responses during examination of human DNA as well as a DNA panel of the above-mentioned microorganisms.

The clinical specificity of **AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal State Institute of Science “Central Research Institute of Epidemiology” of Federal Service for Surveillance on Consumers’ Rights

Protection and Human Well-Being, Moscow, 2010.

2. Guidelines to the **AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT** PCR kit for qualitative detection and differentiation of *Rotavirus A*, *Norovirus* genotype 2, and *Astrovirus* RNA in environmental samples and clinical material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

15. QUALITY CONTROL

In accordance with Federal Budget Institution of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *Rotavirus / Norovirus / Astrovirus*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+*Rotavirus / Astrovirus*** **C+*Norovirus* genotype 2 / STI**	Positive control of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
25.06.11 LA	Cover page, text	The name of Institution was changed to Federal Budget Institution of Science “Central Research Institute for Epidemiology”
27.04.15 ME	Through the text	Corrections according to the template. Grammar corrections
	8.1. RNA Extraction	Information about controls of extraction was added
	8.2.1 Preparing tubes for RT-PCR	Appendix 1 was integrated into the text of the instruction manual as Table 1
	10. Troubleshooting	The section was rewritten
	14. References	The reference to Guidelines was added
21.03.18 PM	3. Content	The color of reagents was specified