AmpliSens® All-screen-FRT PCR kit

Instruction Manual

RUO

For Professional Use Only

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Contains sufficient for <n> tests
RUO	Research Use Only		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C–	Negative control of extraction
IC	Internal control	$C+_{Shig/Salm}$, $C+_{Camp/Adeno}$, $C+_{Rota/Astro}$, $C+_{Noro/STI}$	Positive controls of amplification

1. INTENDED USE

AmpliSens® All-screen-FRT PCR kit is not a medical device. PCR kit is intended for qualitative detection and differentiation of DNA of microorganisms of the *Shigella* complex (*Shigella* spp.) / enteroinvasive *E.coli* (*EIEC*) (without differentiation), salmonella species (*Salmonella* spp.), thermophilic *Campylobacter* spp., *Adenoviruses* group F and RNA of *Rotaviruse*s group A, *Norovirus* genotype 2 (*Norovirus* GII), and *Astroviruses* in the biological material (feces) by real-time hybridization-fluorescence detection of amplified products. The material for RT-PCR is DNA/RNA samples extracted from test material.
PCR kit can be used for examination of environmental objects (water sample concentrates) for carrying out prophylactic measures in order to prevent human diseases.

Indications and contra-indications for use of the reagent kit
The reagent kit is used for the analysis of biological material taken from persons with suspected herpesvirus infection, without distinction of form and presence of disease 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 DNA/RNA extraction from the samples of test material with the exogenous internal control sample (Internal Control-FL (IC)), RNA reverse transcription and simultaneous amplification of DNA/cDNA fragments of the detected microorganisms and cDNA of the internal control with hybridization-fluorescence detection. Exogenous internal control (Internal Control-FL (IC)) allows to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition.
RNA reverse transcription with the Revertase (MMlv) enzyme and amplification of DNA/cDNA fragments with the use of specific primers and Taq-polymerase enzyme are performed with the DNA/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® All-screen-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 using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.
DNA/RNA detection of the microorganisms for a single sample is performed in several test tubes. In each tube 2 pathogens or one pathogen and Internal Control-FL (IC) are differentiated. The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE
Name of PCR-mix	**DNA/cDNA-target**	
PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.	*Shigella* spp. / *EIEC* DNA	*Salmonella* spp. DNA
PCR-mix-1-FEP/FRT *Campylobacter* spp. / *Adenovirus*	*Campylobacter* spp. DNA	*Adenovirus* F DNA
RT-PCR-mix-1-FEP/FRT *Norovirus* / STI	Internal Control-FL (IC) cDNA	*Norovirus* GII cDNA
RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus*	*Rotavirus* A cDNA	*Astrovirus* cDNA
Name of PCR-mix	**Target gene**	
PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.	Ipa H (invasive plasmid antigen)	Ttr (tetrathionate reductase gene)
PCR-mix-1-FEP/FRT *Campylobacter* spp. / *Adenovirus*	23S rRNA	Hexon
RT-PCR-mix-1-FEP/FRT *Norovirus* / STI	Artificially synthesized nucleotide sequence	gene for capsid protein
RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus*	NSP2	gene for capsid protein

3. CONTENT

AmpliSens® All-screen-FRT PCR kit is produced in 2 forms:
variant FRT-50 F, REF R-B45(RG,iQ)-CE;
variant FRT-50 F in bulk[1], REF R-B45(RG,iQ)-CE-B.
Variant FRT-50 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-mix-1-FEP/FRT *Campylobacter* spp. / *Adenovirus*	clear liquid from colorless to light lilac colour	0.6	1 tube
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
PCR-buffer-C	colorless clear liquid	0.3	4 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	4 tubes
TM-Revertase (MMlv)	colorless clear liquid	0.015	4 tubes
RT-G-mix-2	colorless clear liquid	0.015	4 tubes
Positive Control DNA *Shigella sonnei* / *Salmonella* ($C+_{Shigella\ /\ Salmonella}$)	colorless clear liquid	0.1	1 tube
Positive Control DNA *Campylobacter jejuni* / *Adenovirus* F-Flu ($C+_{Campylobacter\ /\ Adenovirus}$)	colorless clear liquid	0.1	1 tube
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
TE-buffer	colorless clear liquid	0.5	1 tube
Internal Control-FL (IC)*	colorless clear liquid	0.6	1 tube
Negative Control (C–)**	colorless clear liquid	1.2	1 tube
Buffer for elution B***	colorless clear liquid	1.2	5 tubes

* add **10 µl** of **Internal Control-FL (IC)** during the extraction procedure directly to the sample/lysis mixture (see **RIBO-sorb**, **RIBO-prep**, and **MAGNO-sorb** protocols).
** must be used in the extraction procedure as Negative control of extraction.
*** must be used in the extraction procedure.
Variant FRT-50 F is intended for 55 tests (220 amplification reactions), including controls.

4. ADDITIONAL REQUIREMENTS

Sampling and pretreatment
- Container (50-60 ml) for sampling, storage and transportation of biological samples, single-use, sterile.
- 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-term storage of biological material (feces) under low-temperature freezing conditions.
- Disposable tightly closed polypropylene 1.5-ml tubes.
- Screw caps for test tubes.
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl).
- Sterile RNase-free pipette tips up to 1000 µl.
- Tube racks.
- PCR box.
- Vortex mixer.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Disposable powder-free gloves and a laboratory coat.
- Reservoir to throw off and inactivate the material.

For DNA/RNA extraction and amplification
- DNA/RNA extraction kit or Automated station for DNA/RNA extraction based on magnetic beads with MAGNO-sorb Nucleic Acid Extraction kit variant 100-200M.
- Set of consumables for used automated station according to the manufacturer's recommendations.
- Sterile RNase-free pipette tips with aerosol filters (up to 100 µl)
- Tube racks.
- PCR box.
- Vortex mixer.
- Disposable polypropylene PCR 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 tubes with caps if a rotor-type instrument is used.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN GmbH, Germany), CFX 96 (Bio-Rad Laboratories, Inc., USA).
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Disposable powder-free gloves and a laboratory coat.
- Reservoir for used tips.

[1] In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section "Content").
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

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

6. SAMPLING AND HANDLING

AmpliSens® All-screen-FRT PCR kit is intended for the analysis of DNA/RNA extracted with DNA/RNA extraction kits from biological material:

- feces samples;
- environmental objects samples (concentrated water samples).

Sampling

Feces samples

Feces samples are taken from a disposable reservoir (for example, a petrie dish, disposable plastic bag) placed in a bed-pan or disposable diapers (for younger children). When using a disposable diaper for children with liquid stool, a cotton swab 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).

Transfer approximately 1 g (or 1 ml) of the sample into a special sterile plastic container using a separate tip with a filter or disposable blades.

Feces samples can be stored before pre-treatment:

- at the temperature from 18 to 25 °C – no more than 6 hours;
- at the temperature from 2 to 8 °C – no more than 3 days;
- at the temperature from minus 24 to minus 16 °C – for 1 week.

Only one freeze-thawing cycle is required.

Water sample concentrates are collected according to state and local authorities' requirements

Water concentrates samples can be stored before the PCR analysis:

- at the temperature from 2 to 8 °C – no more than 1 day;
- at the temperature from minus 24 to minus 16 °C – for 1 month;
- at the temperature not higher than minus 68 °C – for a long time.

Only one freeze-thawing cycle is required.

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

Pretreatment

Pretreatment of w*ater sample concentrates* is not required.

Feces samples are to be pretreated.

Preparation of fecal suspension:

1. Take the required number (respectively to the number of samples) of disposable 1.5-ml tubes. Add 1 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. Use two tips up to 1 ml (with filter and without filter) and a cut lower part of cotton probe (cotton bud) for express filtration.
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 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. Use 100 µl of filtrate for DNA/RNA extraction.

Pretreated fecal suspension samples can be stored before the PCR analysis:

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

Only one freeze-thawing cycle is required.

Pretreated fecal suspension samples can be transported at temperatures from 2 to 8 °C for 1 day.

Interfering substances and limitations of using test material samples

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

Fecal samples obtained directly from a defecation vessel or other reusable container (regardless of their disinfection methods) are incapable for analysis.

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 (see Table 2).

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

Table 2

Type of potential interferent	Potential interferent	Tested concentration in a sample	Interference presence
Endogenous substances	Whole blood	Up to 40% (interferent volume to the sample volume)	Not detected
	Fecal fats	Up to 40% (interferent volume to the sample volume)	Not detected
	Mucin (mucus)	Up to 1% (interferent volume to the sample volume)	Not detected
Exogenous substances	"Enterofuril" oral suspension	Up to 4.25 mg/ml	Not detected
	"Enterosgel", oral paste	Up to 174.75 mg/ml	Not detected
	Dextrin	Up to 68.6 mg/ml	Not detected

7. WORKING CONDITIONS

AmpliSens® All-screen-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. DNA/RNA extraction

NOTE: Only sterile disposable plastic consumables with special RNase-free, DNase-free markings should be used for work with RNA.

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

- **RIBO-prep,**
- **RIBO-sorb,**
- **MAGNO-sorb** (variant 100-200M).

NOTE: Extract DNA/RNA according to the manufacturer's instructions.

MAGNO-sorb nucleic acid extraction kit can be used in combination with "open type" automated nucleic acid extraction stations. DNA/RNA extraction is carried out in accordance with the *Instruction manual* to MAGNO-sorb nucleic acid extraction kit.

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

The volumes of reagents and samples when the DNA/RNA is extracted by RIBO-prep, RIBO-sorb nucleic acid extraction kits:

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–)** to the tube labeled C– (Negative Control of Extraction).

The volume of elution is **50 µl**. It is allowed to increase of the elution volume up to 100 µl.

NOTE: When extracting DNA/RNA from test samples, only **Buffer for elution B** from AmpliSens® AII-screen-FRT PCR kit is used.

The volumes of reagents and samples when the DNA/RNA is extracted by MAGNO-sorb nucleic acid extraction kit:

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

The volume of the test sample:

- when testing feces – **100 µl,**
- when testing water sample concentrates – **200 µl.**

Add **200 µl** of **Negative Control (C–)** to the tube labeled C– (Negative Control of Extraction).

The volume of elution is **100 µl**. It is allowed to increase of the elution volume up to 200 µl.

NOTE: It is recommended to carry out the RT-PCR reaction immediately after obtaining DNA/RNA samples. DNA/RNA samples can be stored before the PCR analysis:
- at the temperature from 2 to 8 °C – no more than 30 min,
- at the temperature from minus 24 to minus 16 °C – no more than 1 week,
- at the temperature not higher than minus 68 °C – up to 1 year.

Only one freeze-thawing cycle is required.

8.2. Preparing reverse transcription and PCR

8.2.1 Preparing tubes for PCR

The total reaction volume is **25 µl**; the volume of DNA/RNA sample is **10 µl**.

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

Use disposable filter tips for adding reagents, DNA/RNA and control samples into tubes.

1. Calculate the required quantity of each reagent for preparation of 4 reaction mixtures. For one reaction:
   - **10 µl** of one of **PCR-mix-1** (**PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.** or **PCR-mix-1-FEP/FRT *Campylobacter* spp. / *Adenovirus*** or **RT-PCR-mix-1-FEP/FRT *Norovirus* / STI** or **RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus***),
   - **5 µl** of **PCR-buffer-C**,
   - **0.5 µl** of **Polymerase (TaqF)**,

   and for "Noro/STI", "Rota/Astro" reaction mixtures:
   - **0.25 µl** of **RT-G-mix-2**,
   - **0.25 µl** of **TM-Revertase (MMlv)**.

Prepare the reaction mixtures for the total number of test and control samples (see numbers of control samples in item 7) plus one extra reaction. The calculation for the required number of reactions can be performed according to Table 3. It is recommended to mix reagents for an even number of reactions for more accurate dosing.

NOTE: The components of reaction mixtures should be mixed immediately before RT-PCR.

2 Thaw the tubes with **PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.**, **PCR-mix-1-FEP/FRT *Campylobacter* spp. / *Adenovirus***, **RT-PCR-mix-1-FEP/FRT *Norovirus* / STI**, **RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus***. Thoroughly vortex contents of all tubes of the PCR kit and sediment the drops by short centrifugation.

3 Prepare four reaction mixtures in four separate test tubes:
   - in the first tube, marked **"Shig/Salm"**, mix the required volume of **PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.**, **PCR-buffer-C, Polymerase (TaqF)**, sediment the drops by short centrifugation,
   - in the second tube, marked **"Camp/Adeno"**, mix the required volume of **PCR-mix-1-FEP/FRT *Campylobacter* spp. / *Adenovirus***, **PCR-buffer-C**, **Polymerase (TaqF)**, sediment the drops by short centrifugation,
   - in the third tube, marked **"Noro/STI"**, mix the required volume of **RT-PCR-mix-1-FEP/FRT *Norovirus* / STI**, **PCR-buffer-C**, **Polymerase (TaqF)**, **RT-G-mix-2**, **TM-Revertase (MMlv),** sediment the drops by short centrifugation,
   - in the fourth tube, marked **"Rota/Astro"**, mix the required volume of **RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus***, **PCR-buffer-C**, **Polymerase (TaqF)**, **RT-G-mix-2**, **TM-Revertase (MMlv),** sediment the drops by short centrifugation.

Table 3

Scheme of reaction mixture preparation

		Reagent volume for specified number of reactions				
Reagent volume for 1 reaction (µl)		**10.00**	**5.00**	**0.25**	**0.50**	**0.25**
The number of test samples	**The number of reactions[2]**	**(RT-)PCR-mix-1-FEP/FRT**	**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**

4. Take the required (four-fold) number of the tubes or strips for RT-PCR of test and control samples. Mark the tubes as **"Shig/Salm"**, **"Camp/Adeno"**, **"Noro/STI",** and **"Rota/Astro"**.
5. Transfer **15 µl** of the prepared **"Shig/Salm"** reaction mixture to each test tube of the first row, **15 µl** of the prepared **"Camp/Adeno"** reaction mixture to each tube of the second row, **15 µl** of the prepared **"Noro/STI"** reaction mixture to each tube of the third row and **15 µl** of the prepared **"Rota/Astro"** reaction mixture to each tube of the fourth row. Discard the unused reaction mixtures.
6. Add **10 µl** of DNA/RNA samples obtained at the extraction stage from the test samples into each four test tubes with different reaction mixtures.

NOTE: Avoid transferring the sorbent together with the DNA/RNA samples extracted with the reagent kit for extraction on silica gel or magnetic separation.

7. Carry out the control amplification reactions:

NCA – Add **10 µl** of **TE-buffer** to each of four tubes with different reaction mixtures marked **"Shig/Salm"**, **"Camp/Adeno"**, **"Noro/STI", "Rota/Astro"**

C+$_{Shig/Salm}$ – Add **10 µl** of **Positive Control DNA *Shigella sonnei* / *Salmonella*** **(C+**$_{Shigella\ /\ Salmonella}$**)** to the tube with **"Shig/Salm"** reaction mixture

C+$_{Camp/Adeno}$ – Add **10 µl** of **Positive Control DNA *Campylobacter jejuni* / *Adenovirus* F-Flu (C+**$_{Campylobacter\ /\ Adenovirus}$**)** to the tube with **"Camp/Adeno"** reaction mixture

C+$_{Noro/STI}$ – Add **10 µl** of **Positive Control cDNA *Norovirus* genotype 2-Flu / STI (C+**$_{Norovirus\ genotype\ 2\ /\ STI}$**)** to the tube with **"Noro/STI"** reaction mixture

C+$_{Rota/Astro}$ – Add **10 µl** of **Positive Control cDNA *Rotavirus*-Flu / *Astrovirus* (C+**$_{Rotavirus\ /\ Astrovirus}$**)** to the tube with **"Rota/Astro"** reaction mixture

C– – Add **10 µl** of **the sample extracted from the Negative Control (C–) reagent** to each of four tubes with different reaction mixtures labeled **"Shig/Salm"**, **"Camp/Adeno"**, **"Noro/STI", and "Rota/Astro"**.

8.2.2 Amplification

1. Create a temperature profile on your instrument as follows (tables 4, 5)[3]:

Table 4

AmpliSens unified amplification program for rotor-type[4] and plate-type[5] 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**	

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 and plate-type instruments

Step	Temperature, °C	Time	Fluorescence detection	Cycles
1	**50**	**30 min**	**–**	**1**
2	**95**	**15 min**	**–**	**1**
3	**95**	**10 s**	**–**	**45**
	60	**25 s**	**FAM, JOE**	
	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 that cause acute intestinal infections, with the possibility of their usage in one instrument simultaneously. If other tests are performed simultaneously, the detection is assigned 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 two channels (see Table 6).

Table 6

(RT-)PCR-mix-1	Channel for fluorophore	
	FAM	**JOE**
PCR-mix-1 FEP/FRT *Shigella* spp. / *Salmonella* spp.	*Shigella* spp./*EIEC* DNA	*Salmonella* spp. DNA
PCR-mix-1-FEP/FRT *Campylobacter* spp. / *Adenovirus*	*Campylobacter* spp. DNA	*Adenovirus* F DNA
RT-PCR-mix-1-FEP/FRT *Norovirus* / STI	Internal Control-FL (IC) cDNA	*Norovirus* GII cDNA
RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus*	*Rotavirus* A cDNA	*Astrovirus* cDNA

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the RNA sample in the corresponding column of the results grid.
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 7).

Table 7

Results for controls

Reaction mixture	Control	Stage for control	*Ct* value in the channel for fluorophore	
			FAM	**JOE**
All mixtures, except "Noro/STI"	**C–**	DNA/RNA extraction	Absent or > boundary value	Absent or > boundary value
"Noro/STI"	**C–**	DNA/RNA extraction	< boundary value	Absent or > boundary value
"Shig/Salm"	**C+**$_{Shig/Salm}$	RT-PCR	< boundary value	< boundary value
"Camp/Adeno"	**C+**$_{Camp/Adeno}$	RT-PCR	< boundary value	< boundary value
"Noro/STI"	**C+**$_{Noro/STI}$	RT-PCR	< boundary value	< boundary value
"Rota/Astro"	**C+**$_{Rota/Astro}$	RT-PCR	< boundary value	< boundary value
All mixtures	**NCA**	RT-PCR	Absent or > boundary value	Absent or > 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*).
Principle of interpretation is the following:

Table 8

Results interpretation for the test samples

***Ct* value in the channel for the fluorophore**		**Result**
FAM	**JOE**	
"Shig/Salm" reaction mixture		
< boundary value	determined or absent	***Shigella* spp./*EIEC* DNA is detected**
determined or absent	< boundary value	***Salmonella* spp. DNA is detected**
absent or > boundary value*	absent or > boundary value*	***Shigella* spp./*EIEC* DNA and *Salmonella* spp. DNA are NOT detected**
"Camp/Adeno" reaction mixture		
< boundary value	determined or absent	***Campylobacter* spp. DNA is detected**
determined or absent	< boundary value	***Adenovirus* F DNA is detected**
absent or > boundary value*	absent or > boundary value*	***Campylobacter* DNA and *Adenovirus* F DNA are NOT detected**
"Noro/STI" reaction mixture		
determined or absent	< boundary value	***Norovirus* GII RNA is detected**
< boundary value or absent or > boundary value*	absent or > boundary value	***Norovirus* GII RNA is NOT detected**
absent or > boundary value	absent or > boundary value	**Invalid***
"Rota/Astro" reaction mixture		
< boundary value	determined or absent	***Rotavirus* A RNA is detected**
determined or absent	< boundary value	***Astrovirus* RNA is detected**
absent or > boundary value*	absent or > boundary value*	***Rotavirus* A RNA and *Astrovirus* RNA are NOT detected**

* The result is interpreted as "pathogen DNA/RNA is NOT detected" if
- *Ct* value determined for 'Noro/STI' reaction mixture in the channel for FAM fluorophore is less than the boundary value;
- *Ct* value for 'Noro/STI' reaction mixture in the channel for FAM fluorophore is absent or determined greater than the boundary value, provided that *Ct* value determined for at least one of the other channels (the channel for JOE fluorophore for "Noro/STI" reaction mixture, channels for FAM and JOE fluorophores for "Shig/Salm", "Camp/Adeno", "Rota/Astro" reaction mixtures) is less than the boundary.

** If the *Ct* value for "Noro/STI" reaction mixture in channels for FAM and JOE fluorophores is absent or determined greater than the boundary value provided that the *Ct* value for "Shig/Salm", "Camp/Adeno", "Rota/Astro" reaction mixtures in channels for FAM and JOE fluorophores is absent or determined greater than the boundary value, the result is considered invalid. If an **invalid result** is obtained, PCR analysis of the corresponding test sample should be repeated starting from DNA/RNA extraction stage.

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

[2] The number of test samples + negative control of DNA/RNA extraction + 2 controls of amplification + 1 extra sample (N+1+2+1, N is the number of test samples).
[3] The amplification programs (tables 4, 5) are equivalent for the use with this PCR kit.
[4] For example, Rotor-Gene 3000/6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany).
[5] For example, CFX 96 (Bio-Rad Laboratories, Inc., USA).

10. TROUBLESHOOTING

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

1. For the Positive Control of RT-PCR (C+):
   a) the *Ct* value for all reaction mixtures (except "Noro/STI" reaction mixture) in the channels for FAM and/or JOE fluorophores and for "Noro/STI" reaction mixture in the channel for the JOE fluorophore is greater than the boundary value or absent. The results interpretation is not possible for the samples in which the specific DNA/RNA was not detected. The amplification should be repeated for these samples. It is necessary to exclude the situation described in paragraph 4 for the samples in which the specific DNA/RNA was detected.
   b) the *Ct* value for "Noro/STI" reaction mixture determined in the channel for the FAM fluorophore is greater than the boundary value or absent. The results interpretation is not possible for the samples in which the specific DNA/RNA was not detected for all analyzed targets (the *Ct* value for all reaction mixtures (except "Noro/STI" reaction mixture) in the channels for FAM and JOE fluorophores and for "Noro/STI" reaction mixture in the channel for JOE fluorophore is greater than the boundary value or absent). The amplification should be repeated for these samples. It is necessary to exclude the situation described in paragraph 4 for the samples in which *Ct* value for "Noro/STI" reaction mixture in the channel for the FAM fluorophore is determined less than the boundary value.
2. For the Negative Control of Extraction (C–):
   a) the *Ct* value for all reaction mixtures (except "Noro/STI" reaction mixture) in the channels for FAM and/or JOE fluorophores and for "Noro/STI" reaction mixture in the channel for the JOE fluorophore is determined 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 specific DNA/RNA was detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the DNA/RNA extraction stage) should be repeated for these samples.
   b) the *Ct* value for "Noro/STI" reaction mixture determined in the channel for the FAM fluorophore is greater than the boundary value or absent. The results interpretation for the test samples should be performed according to the Table 8.
3. For the Negative Control of RT-PCR (NCA):
   a) the *Ct* value for all reaction mixtures (except "Noro/STI" reaction mixture) in the channels for FAM and/or JOE fluorophores and for "Noro/STI" reaction mixture in the channel for the JOE fluorophore is determined less than the boundary value, whereas the area of typical exponential growth of fluorescence is present on the fluorescence graphic. 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 specific DNA/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 for "Noro/STI" reaction mixture determined in the channel for the FAM fluorophore is less than the boundary value, whereas the area of typical exponential growth of fluorescence is present on the fluorescence graphic. 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 specific DNA/RNA was not detected. Measures for detecting and elimination of contamination source must be taken. The amplification should be repeated for these samples.
   c) the *Ct* value is determined but the fluorescence graphic along this channel does not have the correct shape with the area of typical exponential growth of fluorescence (the graphic looks like straight line). The result is erroneous and is interpreted as negative.
4. The *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level or parameters of base line calculation. If the result has been obtained with the correct level of threshold line (base line), the amplification should be repeated for this sample.

11. TRANSPORTATION

AmpliSens® All-screen-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® All-screen-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp., PCR-mix-1-FEP/FRT *Campylobacter* spp. / *Adenovirus*, RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus*, RT-PCR-mix-1-FEP/FRT *Norovirus* / STI, PCR-buffer-C, polymerase (TaqF), TM-Revertase (MMlv), and RT-G-mix-2). All components of the **AmpliSens® All-screen-FRT** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp., PCR-mix-1-FEP/FRT *Campylobacter* spp. / *Adenovirus*, RT-PCR-mix-1-FEP/FRT *Rotavirus* / *Astrovirus*, RT-PCR-mix-1-FEP/FRT *Norovirus* / STI, 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 ºC.

NOTE: PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp., PCR-mix-1-FEP/FRT *Campylobacter* spp. / *Adenovirus*, 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. Analytical sensitivity (limit of detection)

Pathogen	Test material	Analytical sensitivity, GE/ml[6]
Shigella spp. and enteroinvasive *E. coli* (*EIEC*)	feces, concentrated water samples	$1x10^3$
Salmonella spp.	feces, concentrated water samples	$1x10^3$
Thermophilic *Campylobacter* spp.	feces, concentrated water samples	$1x10^3$
Adenovirus F	feces, concentrated water samples	$1x10^4$
Rotavirus A	feces, concentrated water samples	$1x10^4$
Norovirus genotype 2	feces, concentrated water samples	$5x10^3$
Astrovirus	feces, concentrated water samples	$1x10^4$

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® All-screen-FRT** PCR kit is ensured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis.

AmpliSens® All-screen-FRT PCR kit detects the RNA fragments of claimed microorganisms

The analytical specificity was confirmed on the investigating of DNA/RNA of following microorganism/strains and human genomic DNA:

- strains from ATCC (American Type Culture Collection, USA) in concentration at least $1x10^6$ GE/ml: *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC® 14028™, *Salmonella enterica* subsp. *enterica* serovar Choleraesuis ATCC® 10708™, *Salmonella enterica* subsp. *diarizonae* ATCC® 12325™, *Salmonella enterica* subsp. *enterica* serovar Paratyphi C ATCC® 13428™, *Shigella sonnei* ATCC® 25931™, *Campylobacter fetus* subsp. *fetus* ATCC® 27374™, *Campylobacter jejuni* subsp. *jejuni* ATCC® 33560™, *Salmonella bongori* ATCC® 43975™, *Salmonella enterica* subsp. *indica* ATCC® 43976™, *Campylobacter coli* ATCC® 49941™, *Salmonella enterica* subsp. *enterica* serovar Paratyphi B ATCC® 8759™, *Salmonella enterica* subsp. *enterica* serovar Oranienburg ATCC® 9239™, *Shigella boydii* ATCC® 9905™, *Salmonella enterica* subsp. *enterica* serovar Abony ATCC® BAA-2162™, *Campylobacter hominis* ATCC® BAA-381™;
- viral DNA/RNA: Quantitative Genomic DNA from *Human adenovirus* 41 ATCC® VR-930DQ™, Quantitative Synthetic RNA from *Astrovirus* ATCC® VR-3238SD™ in concentration at least $1x10^4$ GE/ml;
- viral DNA/RNA: Quantitative Synthetic DNA from *Hepatitis* A virus ATCC® VR-3257SD™, Quantitative Synthetic *Norovirus* G1 (I) RNA ATCC® VR-3234SD™, Quantitative Synthetic RNA from *Sapovirus* ATCC® VR-3237SD™ in concentration at least $1x10^4$ GE/ml and no more than $1x10^5$ GE/ml;
- strains from ATCC (American Type Culture Collection, USA) in concentration at least $1x10^6$: *Acinetobacter baumannii* ATCC® 19606™, *Aggregatibacter aphrophilus* ATCC® 7901™, *Bacteroides fragilis* ATCC® 25285™, *Bordetella bronchiseptica* ATCC® 10580™, *Bordetella bronchiseptica* ATCC® 4617™, *Bordetella pertussis* ATCC® 9340™, *Candida albicans* ATCC® 14053™, *Candida krusei* ATCC® 14243™, *Clostridioides difficile* ATCC® 9689™, *Clostridium septicum* ATCC® 12464™, *Corynebacterium jeikeium* ATCC® 43734™, *Corynebacterium minutissimum* ATCC® 23348™, *Corynebacterium xerosis* ATCC® 373™, *Cutibacterium acnes* ATCC® 11827™, *Eggerthella lenta* (*Eubacterium lentum*) ATCC® 43055™, *Enterobacter cloacae* subsp. *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™, *Klebsiella aerogenes* ATCC® 13048™, *Klebsiella oxytoca* ATCC® 49131™, *Klebsiella pneumoniae* subsp. *pneumoniae* ATCC® 27736™, *Listeria grayi* (*murrayi*) ATCC® 25401™, *Listeria innocua* ATCC® 33090™, *Listeria monocytogenes* ATCC® 7644™, *Meyerozyma guilliermondii* ATCC® 6260™, *Moraxella* (*Branhamella*) *catarrhalis* ATCC® 25238™, *Moraxella* (*Branhamella*) *catarrhalis* ATCC® 25240™, *Moraxella* (*Branhamella*) *catarrhalis* ATCC® 8176™, *Neisseria gonorrhoeae* ATCC® 49926™, *Neisseria gonorrhoeae* ATCC® 19424™, *Neisseria lactamica* ATCC® 23970™, *Neisseria meningitidis* ATCC® 13102™ (Serogroup C), *Neisseria meningitidis* ATCC® 13090™ (Serogroup B), *Peptostreptococcus anaerobius* ATCC® 27337™, *Proteus mirabilis* ATCC® 12453™, *Proteus vulgaris* ATCC® 6380™, *Pseudomonas aeruginosa* ATCC® 15442™, *Rhodococcus equi* ATCC® 6939™, *Serratia marcescens* ATCC® 14756™, *Staphylococcus aureus* (MRSA) ATCC® 43300™, *Staphylococcus aureus* ATCC® 29213™, *Staphylococcus aureus* ATCC® 25923™, *Staphylococcus aureus* ATCC® 33862™, *Staphylococcus aureus* subsp. *aureus* (MRSA) ATCC® 33591™, *Staphylococcus aureus* subsp. *aureus* ATCC® 12600™, *Staphylococcus aureus* subsp. *aureus* ATCC® 6538P™, *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 dysgalactiae* subsp. *equisimilis* ATCC® 12388™, *Streptococcus equi* subsp. *equi* ATCC® 9528™, *Streptococcus gallolyticus* ATCC® 9809™, *Streptococcus mutans* ATCC® 35668™, *Streptococcus pneumoniae* ATCC® 27336™, *Streptococcus pneumoniae* ATCC® 49619™, *Streptococcus pneumoniae* ATCC® 6303™, *Streptococcus pneumoniae* ATCC® 6305™, *Streptococcus pyogenes* ATCC® 19615™, *Streptococcus salivarius* subsp. *salivarius* ATCC® 13419™, *Streptococcus uberis* ATCC® 700407™, *Trichophyton interdigitale* ATCC® 9533™, *Trichophyton rubrum* ATCC® 28188™, *Vibrio parahaemolyticus* ATCC® 17802™, *Vibrio vulnificus* ATCC® 27562™;
- human DNA in concentration of 0.2 mg/ml.

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

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

13.3. Reproducibility and repeatability

Repeatability and reproducibility were determined by testing of positive and negative model samples. Positive samples were quality control samples (QCS) containing *Shigella* spp., *Campylobacter* spp., *Salmonella* spp., *Adenovirus* F, *Rotavirus*, *Norovirus* GII, *Astrovirus* RNA in concentrations corresponding to the limit of detection. Negative control (C–) reagent 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, %
Shigella spp./*EIEC*	10	100	40	100
Salmonella spp.	10	100	40	100
Campylobacter spp.	10	100	40	100
Adenovirus F	10	100	40	100
Rotavirus A	10	100	40	100
Norovirus GII	10	100	40	100
Astrovirus	10	100	40	100
Negative	10	100	40	100

[6] Genome equivalents of the pathogen agent per 1 ml of the sample.

14. REFERENCES

1. Norovirus and Rotavirus Disease Severity in Children: Systematic Review and Meta-analysis. Riera-Montes M, O'Ryan M, Verstraeten T Pediatr Infect Dis J. 2018 Jun;37(6):501-505.
2. The Vast and Varied Global Burden of Norovirus: Prospects for Prevention and Control. Lopman BA, Steele D, Kirkwood CD, Parashar UD. PLoS Med. 2016 Apr 26;13(4):e1001999.
3. https://www.cdc.gov/norovirus/lab/diagnosis.html
4. Enteric adenoviruses. Mautner V, Steinthorsdottir V, Bailey A. Curr Top Microbiol Immunol. 1995;199 (Pt 3):229-82.

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 **AmpliSens® All-screen-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
02.04.25 HM	Through the text	Corrections according to the template
	1. Intended use	The intended use was specified. The subsection *Indications and contra-indications for use of the reagent kit* was added
	2. Principle of PCR detection	Section was updated
	3. Content	The name of reagents were changed: RT-PCR-mix-2-FEP/FRT (5 tubes) to PCR-buffer-C (4 tubes), DNA-buffer to TE-buffer, RNA-eluent to Buffer for elution B. Internal Control STI-87-rec (IC) (0.12 ml x 5) was changed to Internal Control-FL (IC) (0.6 ml x 1). the volume of Negative Control (C–) was changed from 1.6 ml to 1.2 ml
	4. Additional requirements	The section was actualized and updated with materials and instruments
	5. General precautions	List of precautions was expanded
	6. Sampling and handling	The information about sampling and handling was expanded. The subsection *Interfering substances and limitations of using test material samples* was added
	7. Working conditions	Temperature range was changed. Relative humidity was added
	8. Protocol	Working procedure was rewritten. AmpliSens unified amplification program was added
	9. Data Analysis	Information on the correspondence of the amplification product and channels for the fluorophore, the principle of results interpretation for the test samples and controls are presented in tables
	10. Troubleshooting	The section was rewritten
	12. Stability and storage	Actualization according to changes in the "Content" section
	13. Specifications	The list of microorganisms/strains to prove the analytical specificity was expanded. The subsection *13.3. Reproducibility and repeatability* was added
	14. References	References were renewed

AmpliSens®

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