AmpliSens® *Shigella* spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FRT PCR kit

Instruction Manual

CE IVD

For Professional Use Only

KEY TO SYMBOLS USED

REF	Catalogue number	⚠	Caution
LOT	Batch code	Σ	Contains sufficient for <n> tests
IVD	*In vitro* diagnostic medical device		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
EC REP	Authorized representative in the European Community	**C+**$_{Shigella\ /\ Salmonella}$, **C+**$_{Campylobacter\ /\ STI}$	Positive controls of amplification
EIEC	Enteroinvasive *E.coli*	**IC**	Internal control

1. INTENDED USE

AmpliSens® *Shigella* spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of DNA of *Shigella* species (*Shigella* spp.) and enteroinvasive *E.coli* (*EIEC*), *Salmonella* species (*Salmonella* spp.), and thermophilic *Campylobacter* species (*Campylobacter* spp.) in environmental samples and clinical material using real-time hybridization-fluorescence detection of amplified products. The PCR kit does not differentiate enteroinvasive *E.coli* (*EIEC*) and *Shigella* spp. microorganisms. It is associated with the location of the target gene on the plasmid and the exchange ability of the microorganisms. Bacteriological methods should be used for the differentiation of *EIEC* and *Shigella* spp. microorganisms.

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

2. PRINCIPLE OF PCR DETECTION

Shigella spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp. detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Shigella* spp. and *EIEC*, *Salmonella* spp., and *Campylobacter* spp. primers. In 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® *Shigella* spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control-FL (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® *Shigella* spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-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.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE
Name of PCR-mix	**DNA-target**	
PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.	*Shigella* spp. DNA	*Salmonella* spp. DNA
PCR-mix-1-FEP/FRT *Campylobacter* spp. / STI	*Campylobacter* spp. DNA	Internal Control DNA
Name of PCR-mix	**Target gene**	
PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.	Ipa H (invasion plasmid antigen)	Ttr (thiocyanate reductase gene)
PCR-mix-1-FEP/FRT *Campylobacter* spp. / STI	23SrRNA	Artificially synthesized sequence

3. CONTENT

AmpliSens® *Shigella* spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FRT PCR kit is produced in 1 form:

variant FRT-50 F REF R-B44(RG,iQ)-CE

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. / STI	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.02	2 tubes
DNA-buffer	colorless clear liquid	0.5	1 tube
Positive Control DNA *Shigella sonnei* / *Salmonella* (C+$_{Shigella\ /\ Salmonella}$**)**	colorless clear liquid	0.1	1 tube
Positive Control DNA *Campylobacter jejuni* / STI (C+$_{Campylobacter\ /\ STI}$**)**	colorless clear liquid	0.1	1 tube
Negative Control (C-)*	colorless clear liquid	1.6	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube
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-FL (IC)** during the DNA extraction procedure directly to the sample/lysis mixture (see **DNA-sorb-B** REF K1-2-50-CE, **RIBO-prep** REF K2-9-Et-50-CE).

*** must be used in the extraction procedure.

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

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 20 and up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia), iCycler iQ or iCycler iQ5 (Bio-Rad, USA), or equivalent).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml) when working with PCR kit variant FRT-50 F:
  a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  b) 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.
- 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 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 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.
- 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

NOTE: 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® *Shigella* spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FRT PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the environmental samples (concentrated water samples) and clinical material (faeces samples).
Concentrated water samples are used without treatment.

NOTE: The clinical material must be taken according to state and local authorities' requirements.

NOTE: Liquid feces can be used without the suspension preparation stage.

7. WORKING CONDITIONS

AmpliSens® *Shigella* spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

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

- DNA-sorb-B, REF K1-2-50-CE.
- RIBO-prep, REF K2-9-Et-50-CE.

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

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

NOTE: Use the RNA-eluent only from this kit in the procedure of DNA extraction

8.2. Preparing PCR

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

8.2.1 Preparing tubes for PCR

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

NOTE: Reaction mixture components should be mixed just before analysis with calculating for the required number of reactions (test and control samples) according to Table 1. Note that even for analysis of one test or control DNA sample, it is necessary to carry out all controls of the amplification stage: positive control of amplification (C+), negative control of amplification (NCA). It is recommended to mix the reagents for an even reaction number to ensure more exact dosage.

1. Take the required number of tubes for amplification of DNA extracted from test and control samples. The type of tubes depends on the PCR instrument used for analysis.
2. To prepare the reaction mixture, mix **PCR-mix-1 (PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.** or **PCR-mix-1-FEP/FRT *Campylobacter* spp. / STI)** with **PCR-mix-2-FRT** and **polymerase (TaqF)** (see Table 2). Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

Table 2

Scheme of reaction mixture preparation

		Reagent volume for specified number of reactions (µl)		
Reagent volume per one reaction (µl)		**10.00**	**5.00**	**0.50**
Number of test samples	**Number of reactions[1]**	**PCR-mix-1-FEF/FRT**	**PCR-mix-2-FRT**	**Polymerase (TaqF)**
2	**6**	**60**	**30**	**3.0**
4	**8**	**80**	**40**	**4.0**
6	**10**	**100**	**50**	**5.0**
8	**12**	**120**	**60**	**6.0**
10	**14**	**140**	**70**	**7.0**
12	**16**	**160**	**80**	**8.0**
14	**18**	**180**	**90**	**9.0**
16	**20**	**200**	**100**	**10.0**
18	**22**	**220**	**110**	**11.0**
20	**24**	**240**	**120**	**12.0**
22	**26**	**260**	**130**	**13.0**
24	**28**	**280**	**140**	**14.0**
26	**30**	**300**	**150**	**15.0**
28	**32**	**320**	**160**	**16.0**

3. Transfer **15 µl** of the prepared reaction mixture to each PCR tube.
4. Using tips with aerosol filter, add **10 µl** of **DNA samples** obtained at the DNA extraction stage to the prepared tubes. Dispose of the unused reaction mixture.
5. Carry out the control amplification reactions:

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

C+*Shigella* / *Salmonella* – Add **10 µl** of **Positive Control DNA *Shigella sonnei* / *Salmonella*** to the tube labeled C+*Shigella* / *Salmonella* (Positive Control of Amplification) for **PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.**

C+*Campylobacter* / STI – Add **10 µl** of **Positive Control DNA *Campylobacter jejuni* / STI** to the tube labeled C+*Campylobacter* / STI (Positive Control of Amplification) for **PCR-mix-1-FEP/FRT *Campylobacter* spp. / STI.**

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

NOTE: Avoid transferring sorbent together with the DNA sample in case of extraction by DNA-sorb-B kit.

8.2.2. Amplification

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

Table 3a

Amplification program for rotor-type instruments[2]

Step	Temperature, °C	Time	Fluorescence detection	Cycles
1	**95**	**15 min**	**–**	**1**
2	**95**	**10 s**	**–**	**45**
	60	**25 s**	**FAM/Green, JOE/Yellow**	
	72	**10 s**	**–**	

Table 3b

Amplification program for plate-type instruments[3]

Step	Temperature, °C	Time	Fluorescence detection	Cycles
1	**95**	**15 min**	**–**	**1**
2	**95**	**10 s**	**–**	**45**
	60	**25 s**	**FAM, JOE**	
	72	**10 s**	**–**	

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

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.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in the channels for FAM and JOE fluorophores.

Table 4

Detection channels and the pathogens correspondence table

The channel for the fluorophore	PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.	PCR-mix-1-FEP/FRT *Campylobacter* spp. / STI
FAM	*Shigella* spp. DNA	*Campylobacter* spp. DNA
JOE	*Salmonella* spp. DNA	Internal Control-FL (IC)

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 DNA sample in the corresponding column of the results grid.
The principle of interpretation is given in Table 5.

Table 5

Interpretation of results for PCR-analysis

The channel for fluorophore	PCR-mix-1 FEP/FRT *Shigella* spp. / *Salmonella* spp.	PCR-mix-1-FEP/FRT *Campylobacter* spp. / STI
FAM	< boundary value *Shigella* spp. DNA is detected	< boundary value *Campylobacter* spp. DNA is detected
	absent or > boundary value *Shigella* spp. DNA is not detected[4]	absent or > boundary value *Campylobacter* spp. DNA is not detected[4]
JOE	< boundary value *Salmonella* spp. DNA is detected	< boundary value results are valid
	absent or > boundary value *Salmonella* spp. DNA is not detected[4]	absent or > boundary value results are invalid[5]

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 6).

Table 6

Results for control

PCR-mix-1	Control	Stage for control	*Ct* value in the channel for fluorophore	
			FAM	JOE
PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.	C–	DNA extraction	absent or > boundary value	absent or > boundary value
	NCA	PCR	absent or > boundary value	absent or > boundary value
	C+*Shigella* / *Salmonella*	PCR	< boundary value	< boundary value
PCR-mix-1-FEP/FRT *Campylobacter* spp. / STI	C–	DNA extraction	absent or > boundary value	< boundary value
	NCA	PCR	absent or > boundary value	absent or > boundary value
	C+*Campylobacter* / STI	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 FAM or JOE fluorophores is greater than the boundary *Ct* value, the amplification and detection should be repeated for all samples in which the *Ct* value in the channels for FAM and JOE fluorophores was greater than the boundary *Ct* value for required PCR-mix-1.
2. If the *Ct* value determined for the Negative Control of Extraction (C–) (except the channel for JOE fluorophore for PCR-mix-1 FEP/FRT *Campylobacter* / STI) and/or Negative Control of Amplification (NCA) in the channels for the FAM or JOE fluorophores is less than the boundary *Ct* value, the PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which DNA of respective pathogen was detected.

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

[1] Number of test samples plus the control of DNA extraction, two controls of amplification, and one extra reaction (N+1+2+1, N is the number of test samples).

[2] For example, Rotor-Gene 3000 or Rotor-Gene 6000.

[3] For example, iQ5 or Mx3000P.

[4] If the *Ct* value determined for PCR-mix-1-FEP/FRT *Campylobacter* spp. / STI in the channel for the JOE fluorophore is less than the boundary value.

[5] If the *Ct* value for PCR-mix-1-FEP/FRT *Campylobacter* spp. / STI in the channel for the JOE fluorophore is absent or greater than the boundary value, the negative result using other PCR-mix-1 is invalid. The PCR analysis should be repeated (starting from the DNA extraction stage) for such test sample.

11. TRANSPORTATION

AmpliSens® *Shigella* spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-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® *Shigella* spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-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. / STI, PCR-mix-2-FRT and polymerase (TaqF)). All components of the **AmpliSens® *Shigella* spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FRT** PCR kit are stable until the expiry 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. / STI, PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at temperature from minus 24 to minus 16 °C when not in use.

NOTE: PCR-mix-1-FEP/FRT *Campylobacter* spp. / STI and PCR-mix-1-FEP/FRT *Shigella* spp. / *Salmonella* spp. are to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Pathogen	Test material	DNA extraction kit	PCR kit	Analytical sensitivity, GE/ml[6]
Shigella spp. and enteroinvasive *E.coli* (*EIEC*)	feces	RIBO-prep	PCR kit Variant FRT-50 F	$1x10^3$
Salmonella spp.				
Campylobacter spp.				

13.2. Specificity

The analytical specificity of **AmpliSens® *Shigella* spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FRT** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis. Nonspecific responses were absent during examination of human DNA as well as a DNA panel of the following microorganisms:

GISK collection: *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; *Rhinovirus*; *RS viruses*; and human *Adenovirus* types 3, 5, 7, 37, and 40.

VGNKI collection: *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 aureus* 653 and *S. aureus* 29112; *Morganella morganii* 619 c 01; and *Enterobacter fecalis* 356.
Center for Disease Control and Prevention (CDC, USA) collection: 44 isolates of *Norovirus* genotype 1 and 2 different gene clusters; 40 strains of different *rotavirus* [P]G types, 19 strains of *Astrovirus* serotypes 1, 2, 4, 5, and 8; and 15 strains of different *Adenovirus* types and the following bacterial strains (see Table 7).

Table 7

Panel of bacterial pathogens (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* spp.	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	-	-

The clinical specificity of **AmpliSens® *Shigella* spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-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 Budget Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being.
2. Guidelines to the **AmpliSens® *Shigella* spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FRT** PCR kit for qualitative detection and differentiation of *Shigella* species (*Shigella* spp.) and enteroinvasive *E.coli* (*EIEC*), *Salmonella* species (*Salmonella* spp.), and thermophilic *Campylobacter* species (*Campylobacter* spp.) DNA 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 compliance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® *Shigella* spp. and EIEC / *Salmonella* spp. / *Campylobacter* spp.-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
26.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"
02.04.15 PM	Text	Corrections according to the template. Grammar corrections
	8.1. DNA extraction	Additions about carrying out the control of extraction
	8.2.1. Preparing tubes for PCR	Scheme of reaction mixture preparation was added from Appendix 1
	9. Data analysis	The sections were rewritten
	10. Troubleshooting	
13.03.19 EM	3. Content	The colour of the reagents was specified
20.05.20 EM	Through the text	The text formatting was changed
	Footer	The phrase "Not for use in the Russian Federation" was added
	2. Principle of PCR detection	The table with targets was added
11.03.21 MM	—	The name, address and contact information for Authorized representative in the European Community was changed
20.07.21 EM	1. Intended use	The information about the inability of the PCR kit for microorganism differentiation and the use of bacteriological methods was added

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