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For Professional Use Only

AmpliSens® *Shigella* spp. and *EIEC*-FEP

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *Shigella* spp. and *EIEC*-FEP PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Shigella* spp. and enteroinvasive *E.coli* DNA in clinical material by using end-point 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

Shigella spp. and enteroinvasive *E.coli* DNA detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *Shigella* spp. and enteroinvasive *E.coli* primers. In **F**luorescent **E**nd-**P**oint PCR, the amplified product is detected by using fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect fluorescence emission from the fluorophores in a reaction mixture after PCR. It allows detection of the accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® *Shigella* spp. and *EIEC*-FEP** PCR kit is a qualitative test that contains the Internal Control (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*-FEP** 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). Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *Shigella* spp. and *EIEC*-FEP PCR kit is produced in 1 form:

AmpliSens® *Shigella* spp. and *EIEC*-FEP PCR kit variant FEP-50 F, **REF** B12-FEP-CE.

AmpliSens® *Shigella* spp. and *EIEC*-FEP PCR kit variant FEP-50 F includes:

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-FL *Shigella* spp. / STI	colorless clear liquid	0.6	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
Positive Control DNA *Shigella* sonnei / STI (C+$_{Shigella\ /\ STI}$)	colorless clear liquid	0.1	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 dropper bottle
Negative Control (C-)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube

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

** add 10 µl of Internal Control during the DNA extraction procedure directly to the sample/lysis mixture (DNA-sorb-B, **REF** K1-2-50-CE or RIBO-prep, **REF** K2-9-Et-50-CE).

AmpliSens® *Shigella* spp. and *EIEC*-FEP PCR kit 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 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml tubes.
- PCR box.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), MaxyGene (Axygen, USA), GeneAmp PCR System 2700 (Applied Biosystems, USA), or equivalent).
- Fluorometer (for example, ALA-1/4 (Biosan, Latvia) or equivalent).
- Personal computer.
- Disposable polypropylene microtubes for PCR (0.5- or 0.2-ml) (for example, Axygen,

USA).

- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all samples and unused reagents in compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5 % sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which 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 manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

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

AmpliSens® *Shigella* spp. and *EIEC*-FEP PCR kit is intended for analysis of DNA extracted by using DNA extraction kits from clinical material.

7. WORKING CONDITIONS

AmpliSens® *Shigella* spp. and *EIEC*-FEP 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.

Extract DNA according to the manufacturer's instructions.

8.2. Preparing PCR

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

8.2.1. Preparing tubes for PCR

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

1. Before starting work, thaw and thoroughly vortex all reagents of the kit. Make sure that there are no drops on the caps of the tubes.
2. Take the required number of 0.2- or 0.5-ml tubes for amplification for the clinical and control samples and the **Background** samples. The type of tubes depends on the PCR instrument used for analysis.
3. To prepare the reaction mixture, mix **PCR-mix-1-FL *Shigella* spp. / STI** and **PCR-mix-2-FRT** in a new sterile tube (see Appendix 1). Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

4. Mark two tubes as **Background** and add 15 µl of the prepared mixture (without **Polymerase (TaqF)**) and 10 µl of the **DNA-buffer** to each tube. Mix by pipetting. Add above **1** drop of **mineral oil for PCR** (~**25 µl**).
5. Add **Polymerase (TaqF)** to the remaining reaction mixture (see Appendix 1). Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.
6. Transfer **15 µl** of the prepared reaction mixture to each PCR tube. Add above **1** drop of **mineral oil for PCR** (~**25 µl**).
7. Add **10 µl** of **DNA samples** obtained from the clinical samples. Utilize the rest of reaction mixture.

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

8. Carry out the control amplification reactions:

C+ -Add **10 µl** of **Positive Control DNA *Shigella* sonnei / STI** to the tube labeled C+ (Positive Control of Amplification).

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

Background Add **10 µl** of **DNA-buffer** to the prepared tubes labeled **Background**.

8.2.2. Amplification

Run the following program in the thermocycler (see Table 1). When the temperature reaches 95 °C (pause mode), insert tubes into the thermocycler cells and press the button to continue.

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

Table 1

DNA amplification program

	Thermocyclers with active temperature adjustment			Thermocyclers with block temperature adjustment		
	GeneAmp PCR System 2700 (Applied Biosystems), Gradient Palm Cycler (Corbett Research), MaxyGene (Axygen, USA)			**Uno-2 (Biometra)**		
Step	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
0	**95**	pause		**95**	pause	
1	**95**	15 min	1	**95**	15 min	1
2	**95**	10 s	42	**95**	1 min	42
	60	25 s		**60**	1 min	
	72	25 s		**72**	1 min	
3	**72**	1 min	1	**72**	1 min	1
4	**10**	storage		**10**	storage	

Amplification programs for different thermocycler models are described in Guidelines “End-Point PCR Detection of *Shigella* spp. DNA” [2].

9. DATA ANALYSIS

Detection is performed using a fluorescence detector.

Please read the fluorescence detector Operating Manual before using this kit.

Program the detector according to the manufacturer's manual and Guidelines [2].

The fluorescent signal intensity is detected in two channels:

– the signal from the IC DNA amplification product is detected in the FAM channel (or analogous, depending on the detector model);

– the signal from the *Shigella* spp. DNA amplification product is detected in the HEX channel (or analogous, depending on the detector model).

Prior to detection, all settings should be entered and saved. Refer to the **Guidelines** and the **Important Product Information Bulletin** for settings.

Principle of interpretation is given in the table 2.

Table 2

Interpretation of amplification results

Ct value in channel		**Interpretation**
FAM	**HEX**	
> threshold or **<** threshold	**>** threshold of positive result	*Shigella* spp. DNA is **detected**
> threshold	**<** threshold of negative result	*Shigella* spp. DNA is **not detected**
< threshold	**<** threshold of negative result	**Invalid result**
> threshold	**>** threshold of negative result or **<** threshold of positive result	**Equivocal result**

If the result is invalid or equivocal, the PCR should be repeated once again.

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 (Table 3).

Table 3

Results for controls

Control	Stage for control	Result of automatic interpretation		Interpretation
		FAM channel	HEX channel	
C–	DNA extraction	**>** threshold	**<** threshold of negative result	**OK**
NCA	Amplification	**<** threshold	**<** threshold of negative result	**OK**
C+	Amplification	**>** threshold	**>** threshold of positive result	**OK**

10. TROUBLESHOOTING

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

1. If the signal of the Positive Control of amplification (C+) in the HEX channel is less than the threshold of positive result, PCR and detection should be repeated for all samples in which *Shigella* spp. DNA was not detected.
2. If the signal of the Negative Control of extraction (C-) and/or amplification (NCA) detected in the HEX channel is greater than the threshold of positive signal, PCR analysis should be repeated (starting from DNA extraction) for all samples in which *Shigella* spp. DNA was detected.
3. Positive result obtained for Negative Control of extraction (C-), that is a sterile sample of the culture medium, may indicate contamination of the primary enrichment medium with the genetic material of the examined microorganism. In this case, the analysis should be repeated. To do this, start from primary enrichment of food with non-contaminated media and perform an additional negative control extraction reaction using the Negative Control (C–) reagent (see Section 3. Content).
4. If no signal is detected for the positive controls of amplification, it may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components did not comply with the manufacturer’s instruction, or that the reagent kit expired. Programming of the used instrument, storage conditions, and the expiration date of the reagents should be checked, and then PCR should be repeated.
5. No positive signal in C+ may indicate incorrect programming of the temperature profile of the thermocycler, incorrect configuration of PCR, noncompliance of the storage conditions for kit components with the manufacturer's instruction, or the expiration of the reagent kit. Check programming of the thermocycler (see 8.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR once again for all samples.
6. If no signal was detected either in the channel for detection of the pathogen DNA or in

the channel for detection of IC, the sample should be examined once again (PCR and detection). The same applies to the samples with equivocal results, because the fact that the specific signal does not exceed the threshold value is not sufficient to consider a sample as positive. If equivocal results are obtained in the second run, the analysis should be repeated starting from the DNA extraction stage.

7. Positive signal in C– and NCA indicates reagent or sample contamination. In this case, the results of analysis must be considered as invalid. The analyses must be repeated and measures for detecting and eliminating the contamination source must be taken.

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

11. TRANSPORTATION

AmpliSens® *Shigella* spp. and *EIEC*-FEP 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*-FEP** PCR kit (except for PCR-mix-1-FL *Shigella* spp. / STI, polymerase (TaqF), and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Shigella* spp. and *EIEC*-FEP** 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.

PCR-mix-1-FL *Shigella* spp. / STI, polymerase (TaqF), and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 °C when not in use.

PCR-mix-1-FL *Shigella* spp. / STI is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Shigella* spp. and *EIEC*-FEP** PCR kit is the following:

Clinical material	Nucleic acid extraction kit	Sensitivity, GE/ml [1]
Selenite F Broth [2]	DNA-sorb-B	$1x10^3$
	RIBO-prep	$1x10^3$

[1] Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.
[2] Pretreatment is not required.

13.2. Specificity

The analytical specificity of **AmpliSens® *Shigella* spp. and *EIEC*-FEP** 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. Nonspecific reactions were absent while testing human DNA samples and DNA panel of the following microorganisms: 12 strains of different species and serogroups of *Shigella* spp., 31 strains of different serogroups of *Esherichia coli* (including *EHEC, EPEC, ETEC, EAggEC* and *EIEC*), 3 strains of *Cronobacter sakazakii*, 4 strains of *Enterobacter cloacae,* 2 strains of *Enterobacter aerogenes*, 2 strains of *Pantoea agglomerans*, 8 strains of *Campylobacter spp (C. jejuni, C. coli* and *C. fetus fetus)*, 18 strains of different serogroups of *Salmomella* spp., 22 strains of different species and serogroups of *Yersinia* spp., *Citrobacter freundii, Clostridium perfringens, Klebsiella pneumonia, Listeria monocytogene, Protrus mirabilis, Pseudomonas aeruginosa, Serratia marcessens.* The clinical specificity of **AmpliSens® *Shigella* spp. and *EIEC*-FEP** 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, Moscow, 2008.
2. Guidelines “End-Point PCR Detection of *Shigella* spp. DNA”, 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, Moscow.

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® *Shigella* spp. and *EIEC*-FEP** 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+**	Positive control of amplification
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**IC**	Internal control

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”