CE

IVD

For Professional Use Only

AmpliSens® *Vibrio cholerae*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *Vibrio cholerae*-FRT PCR kit is an *in vitro* nucleic acid amplification test for detection of *Vibrio cholerae* DNA and identification of pathogenic strains of *Vibrio cholerae* in the biological material and environmental samples 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

Vibrio cholerae DNA detection (by the presence of the *Hly* sequence), identification of pathogenic *Vibrio cholerae* strains (by the presence of the main virulence factors, *CtxA* and *tcpA*), and species identification to serogroups O1 (by the presence of amplification of the *wbeT* target) and O139 (by the presence of amplification of the *wbfR* target) by the polymerase chain reaction (PCR) are based on the amplification of the pathogen genome specific region using specific *Vibrio cholerae* primers. 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® *Vibrio cholerae*-FRT PCR kit is a qualitative test that contains the Internal Control **(Internal Control *Vibrio cholerae* (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® *Vibrio cholerae*-FRT PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase with a wax layer. Wax melts and reaction components mix only at 95 °C.

3. CONTENT

AmpliSens® *Vibrio cholerae*-FRT PCR kit is produced in 1 form:

AmpliSens® *Vibrio cholerae*-FRT PCR kit variant FRT **REF** R-B53(RG)-CE.

AmpliSens® *Vibrio cholerae*-FRT PCR kit variant FRT includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FRT *Vibrio cholerae* screen ready-to-use single-dose test tubes (*under wax*)	colorless clear liquid	0.008	55 tubes of 0.2 ml
PCR-mix-1-FRT *Vibrio cholerae* type ready-to-use single-dose test tubes (*under wax*)	colorless clear liquid	0.008	55 tubes of 0.2 ml
PCR-mix-2-FL	colorless clear liquid	0.77	1 tube
Positive Control DNA *Vibrio cholerae* screen (C+$_{V.cholerae\ screen}$)	colorless clear liquid	0.1	1 tube
Positive Control DNA *Vibrio cholerae* type (C+$_{V.cholerae\ type}$)	colorless clear liquid	0.1	1 tube
Positive Control IC	colorless clear liquid	0.1	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless clear liquid	1.6	2 tubes
Internal Control *Vibrio cholerae* (IC)**	colorless clear liquid	0.5	1 tube

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

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

AmpliSens® *Vibrio cholerae*-FRT PCR kit is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Sodium merthiolate, 0.1 % solution. Dissolve 0.1 g of sodium merthiolate in 100 ml of 0.9 % NaCl solution to obtain 0.1 % sodium merthiolate solution. This solution should be stored in a black bottle at 2–8 °C for no more than 3 months.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (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 or Rotor-Gene 6000 (Corbett Research, Australia)).

- Disposable polypropylene 0.2-ml PCR tubes (for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from 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 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 manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Vibrio cholerae*-FRT PCR kit is intended for analysis of DNA extracted with DNA extraction kits from biological material and environmental objects.

***6.1 Sampling*:**

Clinical material samples:

- 1.0–2.0 g (or 1-2 ml in case of diarrhea) of feces, native or transferred into tube with 5 ml of 1 % peptone water, are used after pretreatment;
- 1–2 ml of vomit masses, native or transferred into 5 ml of peptone water, are used after pretreatment;
- Rectal swabs taken with a rectal cotton swab from a depth of 5-6 cm (rectal metal snare) should be placed to a 1.5-ml tube containing 0.5 ml of 1 % peptone water. Mix the contents of the tube thoroughly, press the cotton swab against the tube wall, and then discard it into a container with a disinfectant. Thus obtained solution is used for analysis (50 µl).

Autopsy material samples:

- The content (0.5 ml) of the upper, medial, and lower sections of the small intestine are transferred to empty bacteriological tubes (in this case, they are analyzed as native feces) and to tubes with 5 ml of 1 % peptone water (in this case, they are analyzed as cultivated material).

Environmental samples (for monitoring):

- water (from water body, wastewater, or drinking water) is sampled and treated in compliance with local authorities requirements. First peptone water (after pretreatment) is used for analysis;
- silt and aquatic organisms are sampled and treated in compliance with local authorities' requirements. First peptone water (after pretreatment) is used for analysis.

Environmental samples (focus of infection):

- water (from water body, wastewater, drinking water) is sampled and treated in compliance with local authorities' requirements. Then, it is filtered first through filters with a pore diameter of 8 µm (or paper filters) and finally filtered through filters with a pore diameter of 0.45 µm. Filters should be ground and then placed into sterile 10- or 15-ml tubes with 5 ml of 0.9% NaCl. Tubes are agitated on a shaker for 10 min. For PCR analyses, transfer 1.0 ml of the solution into tubes with sealing caps and

centrifuge at 12,000 rpm for 10 min. Resuspend the pellet in 100 µl of 0.9 % NaCl.

If the result of analysis is negative, washing fluids from filters should be used as an inoculum for seeding in compliance with local authorities' requirements and the first peptone water test should be tested (after pretreatment).

— Washing fluids from surfaces of objects (10 x 10 cm area), sampled with a sterile probe wetted in saline (the working part of the probe with the tampon is to be placed to a 1.5-ml tube with 0.5 ml of 1 % peptone water, the rest part of the probe should be broken and discarded). 50 µl of solution without pretreatment is used for analysis.

Food products: are sampled and treated in compliance with local authorities' requirements. First peptone water (after pretreatment) is used for analysis.

***Vibrio cholerae*-suspect cultures of microorganisms**:

— A colony should be resuspended in 0.5 ml of saline or phosphate buffer. 50 µl of suspension is used for analysis.

Material transportation and storage conditions: at ambient temperature for 2 h, at 2–8° C for 1 day, and at minus 24 to minus 16° C for a long time.

The material to be analyzed is transported in strict compliance with local authorities' requirements.

Only one freeze–thaw cycle of biological material is allowed.

***6.2* Pretreatment:**

Native feces:

A. 10-20 % feces suspension preparation (watery feces are used without suspension preparation).

1. 4 ml of saline or phosphate buffer should be transferred to 5-ml tubes with tightly closed cap.
2. 0.5–1.0 g (~ 1–2 ml) of feces are transferred to the tubes using individual tips with aerosol barriers (or disposable spatula) for each tube. The content of the tube should be mixed thoroughly to obtain a homogeneous suspension.

B.1. Preparation of fecal bacterial fraction (for solid feces):

1 ml of the contents of tubes with fecal suspension should be transferred to 1.5-ml tube with tightly closed cap and centrifuged at 12000 rpm for 5 min. For DNA extraction 50 µl of light fraction from the board of transparent liquid and solid fecal fractions is to be used.

B.2. Preparation of fecal bacterial fraction (for watery feces):

1 ml of the contents of tubes with fecal suspension should be transferred to a 1.5-ml tube with a tightly closing cap and centrifuged at 12000 rpm for 5 min. Discard the supernatant using a new tip for each sample, leaving 100–150 µl of the solution above the pellet. Resuspend the pellet in this solution. Thus obtained suspension should be used for DNA extraction.

Feces or vomit masses placed into 1 % peptone water:

A. Mix thoroughly the contents of the tubes to obtain a homogeneous suspension.
B. Bacterial fraction preparation:

   1 ml of the suspension should be transferred to a 1.5-ml tube with a tightly closed cap and centrifuged at 12000 rpm for 5 min. For DNA extraction, 50 µl of the clarified fraction taken at the interface of the liquid transparent and dark solid fractions should be used.

Autopsy material samples (small intestine contents):

Mix thoroughly the content of the tubes to form the homogeneous suspension. For DNA extraction 50 µl of suspension is to be used.

Primary or secondary enrichment medium (after cultivation):

1.0 ml is sampled from the surface of peptone water into the 1.5-ml tube and centrifuged at 12,000 rpm for 10 min. Remove the supernatant using tips with aerosol filters. Pellet is to be resuspended in 300 µl of saline or phosphate buffer. 50 µl of solution is used for analysis.

6.3 Disinfection

See section 8.1.1

7. WORKING CONDITIONS

AmpliSens® *Vibrio cholerae*-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.

Extract DNA according to the manufacturer's instructions.

8.1.1. Sample disinfection

Disinfection is performed in accordance with local authorities' requirements.

Sodium merthiolate is added to the prepared samples (dilution, 1:10000; final concentration, 0.01 %), followed by incubation at 56 °C for 30 min. Then, the required aliquot is added to **Lysis Solution** (component of the **DNA-sorb-B** kit, the order of treatment is specified in Section 8.1.2) or **Solution for Lysis** (component of the **RIBO-prep** kit, the order of treatment is specified in Section 8.1.3). Material is considered disinfected after incubation at 65 °C for 15 min.

8.1.2 DNA extraction with DNA-sorb-B

The volume of the sample for DNA extraction is 0.05 ml.

After adding **300 µl** of **Lysis Solution**, transfer **50 µl** of **Negative Control (C-)** and **50 µl** of samples (disinfected in accordance with Section 8.1.1) into the tubes using tips with aerosol filters.

- After adding **100 µl** of **Negative Control (C-) reagent**, centrifuge the tubes for 5 s to be sure there are no drops on the caps. Then incubate them at 65 °C for 15 min.
- Add **10 µl** of **Internal Control *Vibrio cholerae* (IC)** to each tube, mix the contents of the tubes and then incubate at 65 °C for 5 min.
- Centrifuge the tubes at 8,000–10,000 g (10,000–13,000 rpm, 70 mm radius rotor) for 5 min and transfer the supernatant to a clean tube for subsequent DNA extraction.

After adding 25 **µl** of **Universal Sorbent**, vortex the tubes and leave them in a tube rack for 5 min. Repeat this procedure once again.

Centrifuge tubes at 8,000–10,000 g (10,000–13,000 rpm, 70 mm radius rotor) for 30 s and remove the supernatant from each tube using a vacuum aspirator. Use a new tip for every tube.

Add **300 µl** of **Washing Solution 1** to each tube. Vortex vigorously until the sorbent is completely resuspended. Centrifuge at 8,000–10,000 g (10,000–13,000 rpm, 70 mm radius rotor) for 30 s. Remove the supernatant from each tube using a vacuum aspirator. Use a new tip for each tube.

8.1.3 DNA extraction with RIBO-prep

The volume of the sample for DNA extraction is 0.10 ml.

Add **100 µl** of samples into the tubes with **Solution for Lysis** (disinfected in accordance with Section 8.1.1) using tips with aerosol filters. Add **100 µl** of **Negative Control (C-) reagent** to the tube labeled **C–**.

After incubation, add **10 µl** of **Internal Control *Vibrio cholerae* (IC).** Mix the contents of the tubes thoroughly by vortexing and centrifuge for 5 s to be sure there are no drops on the cap. Then incubate at 65 °C for 5 min. If suspended particles (incompletely dissolved material) are noticed, centrifuge the tubes at 10,000 rpm for 1 min and transfer the supernatant into other tubes.

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

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

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

1. Prepare the required number of the tubes with **PCR-mix-1-FRT *Vibrio cholerae* screen** and **PCR-mix-1-FRT *Vibrio cholerae* type** for DNA amplification of test and control samples and mark the tubes as “T” and “C”, respectively.
2. Add **7 µl** of **PCR-mix-2-FL** to the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FRT**.
3. Using tips with aerosol filter, add **10 µl** of **DNA samples** obtained at the DNA extraction stage.

Avoid transferring the sorbent together with the DNA samples extracted by DNA-sorb-B kit.

4. Carry out the control amplification reactions:

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

C+*V.cholerae* screen – Add **10 µl** of **Positive Control DNA *Vibrio cholerae* screen (C+*V.cholerae* screen)** to the tube with **PCR-mix-1-FRT *Vibrio cholerae* screen** labeled **C+*V.cholerae* screen** (Positive Control of Amplification).

C+*V.cholerae* type – Add **10 µl** of **Positive Control DNA *Vibrio cholerae* type (C+*V.cholerae* type)** to the tube with **PCR-mix-1-FRT *Vibrio cholerae* type** labeled **C+*V.cholerae* type** (Positive Control of Amplification).

IC+ – Add **10 µl** of **Positive Control IC** to the tube with **PCR-mix-1-FRT *Vibrio cholerae* screen** labeled **IC+** (Positive Control of Amplification).

8.2.2. Amplification

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

Table 1

Amplification program for rotor-type instruments[1]

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycles*
Hold	95	5 min	–	1
Cycling	95	10 s	–	10
	60	25 s	–	
	72	10 s	–	
Cycling 2	95	10 s	–	35
	56	25 s	FAM, JOE, ROX	
	72	10 s	–	

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

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

It is necessary to place a test tube into well No. 1.

If “screen” and ”type” tests are performed simultaneously, calibration should be performed using the tube marked NCA that contains PCR-mix-1-FRT *Vibrio cholerae* screen (insert it into well No. 1 of the rotor).

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

9. DATA ANALYSIS

Perform data analysis separately for each PCR-mix-1 by selecting the required tubes.

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in three channels depending on the PCR-mix-1 used:

PCR-mix-1-FRT *Vibrio cholerae* screen

- The signal of the amplification product of *CtxA* gene DNA fragment is detected in the channel for FAM fluorophore;
- The signal of the amplification product of Internal Control DNA is detected in the channel for JOE fluorophore;

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

- The signal of the amplification product of *tcpA* DNA fragment is detected in the channel for ROX fluorophore.

PCR-mix-1-FRT *Vibrio cholerae* type

- The signal of the amplification product of *wbeT* (identifying O1 serogroup) DNA fragment is detected in the channel for FAM fluorophore;
- The signal of the amplification product of *Hly* (all *Vibrio cholerae* serogroups) DNA fragment is detected in the channel for JOE fluorophore.
- The signal of the amplification product of *wbfR* (identifying of O139 serogroup) DNA fragment is detected in the channel for ROX fluorophore.

Results are interpreted by the crossing (or not-crossing) ~~of~~ 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 cDNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

- The sample is considered as **positive** for the target DNA if the *Ct* value determined in the result grid in the channel for the appropriate fluorophore (for example, FAM: ***Quant. Result – Cycling A. FAM***) is less than the boundary *Ct* value specified in the *Important Product Information Bulletin*.
- The samples is considered as **negative** for target DNA if the *Ct* value is not determined (absent) in the result grid in the channel for the appropriate fluorophore (the fluorescence curve does not cross the threshold line).

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 both Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Tables 2 and 3).

Table 2

Results for controls with PCR-mix-1-FRT *Vibrio cholerae* screen

Control	Stage for control	*Ct* value in the channel for the fluorophore		
		FAM (*CtxA*)	JOE (IC)	ROX (*tcpA*)
C–	DNA extraction	absent	<boundary value*	absent
NCA	Amplification	absent	absent	absent
C+*V.cholerae* screen	Amplification	<boundary value*	absent	<boundary value*
IC+	Amplification	absent	<boundary value*	absent

Table 3

Results for controls with PCR-mix-1-FRT *Vibrio cholerae* type

Control	Stage for control	*Ct* value in the channel for the fluorophore		
		FAM (O1)	JOE (*V.cholerae*)	ROX (O139)
C–	DNA extraction	absent	absent	absent
NCA	Amplification	absent	absent	absent
C+$_{V.cholerae\ type}$	Amplification	<boundary value*	<boundary value*	<boundary value*

The results are interpreted according to the Table 4, Guidelines and the *Important Product Information Bulletin* enclosed to the PCR kit

Table 4

Interpretation of results for PCR-analysis

Variants	PCR-mix-1-FRT *Vibrio cholerae* screen			PCR-mix-1-FRT *Vibrio cholerae* type		
	Ct value in the channel for the fluorophore					
	FAM (CtxA)	JOE (IC)	ROX (tcpA)	FAM (O1)	JOE *(V.cholerae)*	ROX (O139)
V.cholerae O1 toxigenic	<boundary value	Any value or its absence	<boundary value	<boundary value	<boundary value	absent
V.cholerae O139 toxigenic	<boundary value	Any value or its absence	<boundary value	absent	<boundary value	<boundary value
V.cholerae O1 NON toxigenic, but contained the sequence **tcpA**	absent	<boundary value	<boundary value	<boundary value	<boundary value	absent
V.cholerae O139 NON toxigenic, but contained the sequence **tcpA**	absent	<boundary value	<boundary value	absent	<boundary value	<boundary value
V.cholerae O1 NON toxigenic	absent	<boundary value	absent	<boundary value	<boundary value	absent
V.cholerae O139 NON toxigenic	absent	<boundary value	absent	absent	<boundary value	<boundary value
V.cholerae not O1 and not O139	absent	<boundary value	absent	absent	<boundary value	absent
Comma bacillus are not detected	absent	<boundary value	absent	absent	absent	absent

10. TROUBLESHOOTING

1. If the *Ct* value is absent in the channels for FAM and ROX fluorophores and the *Ct* value is absent or greater than the boundary *Ct* value in the channel for JOE fluorophore for the samples with the PCR-mix-1-FRT *Vibrio cholerae* screen, the PCR analysis and DNA extraction should be repeated.

2. If the result is positive for any target except for *Hly* target (negative result in the channel for JOE fluorophore in the samples with the PCR-mix-1-FRT *Vibrio cholerae* type) and the *Ct* value determined in the channel for JOE fluorophore is less than the boundary *Ct* value (in the samples with the PCR-mix-1-FRT *Vibrio cholerae* screen), the result of analysis is considered to be invalid. It is recommended to repeat the sample sampling and PCR analysis.
3. If the *Ct* value is absent in the channel for JOE fluorophore for the samples with PCR-mix-1-FRT *Vibrio cholerae* type, provided that the conditions of item 1 are met, the PCR analysis (beginning with the DNA/RNA extraction stage) should be repeated.
4. If the *Ct* value is determined for Negative Control of Extraction (C-) (in the channels for FAM and ROX fluorophores – for PCR-mix-1-FRT *Vibrio cholerae* screen and/or in any of the channels – for PCR-mix-1-FRT *Vibrio cholerae* type) or for Negative Control of Amplification (NCA) (in any of the channels) – this may suggest the contamination of reagents or the samples. In this case, the results of the analysis samples positive in the given channel are considered invalid. Measures for detecting and elimination of contamination source must be taken. The PCR analysis should be repeated for all positive samples.
5. If no signal is detected for the positive controls of amplification, it may suggest that the amplification program was incorrectly chosen, or about other mistakes at PCR stage The PCR-analysis should be repeated.

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

11. TRANSPORTATION

AmpliSens® *Vibrio cholerae*-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® *Vibrio cholerae*-FRT** PCR kit are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Vibrio cholerae*-FRT** PCR kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FRT *Vibrio cholerae* screen and PCR-mix-1-FRT *Vibrio cholerae* type are to be kept away from light.

13. SPECIFICATIONS

13.1. Analytical sensitivity

Biological material	RNA/DNA extraction kit	Analytical sensitivity
Native feces	**DNA-sorb-B** for all material types, **RIBO-prep** for watery feces	1×10^3 GE/ml[2] 1×10^3 m.c./ml[3]
Rectal swabs		
Vomit masses		
Autopsy material		
Water after preliminary filtration		
Washing fluids from environmental samples		
Peptone water after bacterial inoculation or food products		
Germ cultures		

The claimed analytical features of **AmpliSens® *Vibrio cholerae*-FRT** PCR kit are guaranteed only when additional reagents kits **DNA-sorb-B** and **RIBO-prep** (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology") are used.

13.2. Analytical specificity

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

The specific activity of the reagent kit was confirmed in studies of the following reference strains of *V.cholerae*:

- *V.cholerae* strains P-1, KM-569, 10588, KM 26, M045;
- 17 field isolates of *V.cholerae* serogroup O1 isolated in 1991, 1994 and 1999;
- 15 field isolates of *V.cholerae* of other serogroups isolated in 2000, 2001 and 2002 (collection of the Ukrainian Antiplague Station);
- 42 isolates obtained from patients and environmental samples in 1965–2004 (State Collection of Pathogenic Bacteria of the Russian Federation, Mikrob Russian Antiplague Research Institute).

The absence of cross-reactivity during classification into serogroups O1 and O139 was demonstrated for *V.cholerae* strains of different serogroups (O2-O9, O11-O14, O16-O33, O35, O36, O39-O63, O65-O69, O71, O73-O75, O77, O79-O82) from the State Collection of Pathogenic Bacteria of the Russian Federation (Mikrob Russian Antiplague Research Institute).

[2] Genome equivalents of microorganism per 1 ml of the sample from transport medium.
[3] Microbial cells of microorganism per 1 ml of the sample.

The absence of nonspecific reactions of components of the PCR kit was demonstrated for DNA of closely related microorganisms, microorganisms representative of normal microflora, and some other pathogens causing intestinal infections, namely: *Vibrio parahaemolyticus, V.alginolyticus, V.anguillarum, V.mimicus, V.splendidus, V.fluvialis, and V.proteolyticus; Escherichia coli; Salmonella enteritidis* and *S.typhi; Shigella flexneri* and *Sh.sonnei; Campylobacter fetus* and *C.jejuni; Klebsiella pneumonia; Listeria monocytogenes; Proteus vulgaris; Pseudomonas aeruginosa; Staphylococcus aureus; Morganella morganii, Enterobacter faecalis; Aeromonas; Plesiomonas shidel; Commomonas; and* human cDNA/DNA.

False-positive results were not detected in the study of 100 fecal samples without enteritis and 50 fecal samples with enteritis of bacterial and viral etiology.

The clinical specificity of **AmpliSens® *Vibrio cholerae*-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, Moscow, 2010.
2. Guidelines to the **AmpliSens® *Vibrio cholerae*-FRT** PCR kit for detection of *Vibrio cholerae* DNA and identification of pathogenic strains of *Vibrio cholerae* in biological material and environmental samples by 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 **AmpliSens® *Vibrio cholerae*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Expiration Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of Extraction
	Date of manufacture	**C+*V.cholerae* screen, C+*V.cholerae* type**	Positive Controls of Amplification
EC REP	Authorised representative in the European Community	**IC**	Internal control
	Caution	**IC+**	Positive Control IC

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
15.12.10	Cover page	The phrase “For Professional Use Only” was added
	Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” was added.
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of open reagents was added
		Information that PCR-mix-1-FEP/FRT *Vibrio cholerae* type and PCR-mix-1-FEP/FRT *Vibrio cholerae* screen are to be kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
	Text	The name of PCR-mix-1 was changed from PCR-mix-1-FEP/FRT to PCR-mix-1-FRT
		Shorten names of control samples were changed: $C+_{V.cholerae\ screen}$ and $C+_{V.cholerae\ type}$ instead o $C+_{screen}$ и $C+_{type}$, respectively
	6.Sampling and Handling	The description of pretreatment of watery feces and the procedure of DNA extraction from watery feces with the RIBO-prep reagent kit were added
	8.1 DNA extraction	RIBO-prep reagent kit is recommended for DNA extraction
	8.2.2 Amplification	If “screen” and ”type” tests are performed simultaneously, calibration should be performed using the tube marked NCA that contains PCR-mix-1-FRT *Vibrio cholerae* screen
	9. Data analysis	Boundary Ct values are specified in the Important Product Information Bulletin
	13.1 Sensitivity	Analytical specificity table is added
	13.2 Specificity	The information about the absence of nonspecific reactions and cross-reactivity was added
07.12.11 VV	Sampling and handling	The procedure of taking clinical material was refined
	Additional requirements	Sodium merthiolate was added
	Throughout the text	Section describing disinfection of samples was added
		The description of extraction procedure with reagent kits DNA-sorb-B and RIBO-prep was added
20.12.13 GA	Text	“Clinical material” was renamed to “biological material”
		The names of channels of fluorophores were corrected
	4. Additional requirements	Phrase “for ≤ – 16 °C” was changed to “at 24 to minus 16 °C.”
	6. Sampling and handling	
23.10.17 PM	Throughout the text	Corrections according to the template
	10. Troubleshooting	The section was rewritten
	14. References	The reference to the Guidelines was added.