AmpliSens® *Yersinia pestis*-FRT PCR kit

IVD

For Professional Use Only

Instruction Manual

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+	Positive control of amplification
		IC	Internal control

1. INTENDED USE

AmpliSens® *Yersinia pestis*-FRT PCR kit is an *in vitro* nucleic acid amplification test for detection of *Yersinia pestis* DNA in human biological material (blood; bubo aspirate, vesicle aspirate, pustule aspirate, carbuncle aspirate; sputum; oropharyngeal swabs; urine; faeces; lymph nodes; liver, spleen, lungs, adrenal, and brain tissues; as well as pathologically modified tissues and organs), animal material (blood, faeces, parenchymal organs, brain tissues, and pathologically changed tissues and organs), fleas, ticks, bird pellets and soil using real-time fluorescence-hybridization detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

Yersinia pestis DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Yersinia pestis* 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 the 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® *Yersinia pestis*-FRT 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® *Yersinia pestis*-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). 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
DNA-target	Internal Control STI-87 DNA	*Yersinia pestis* DNA
Target gene	Artificially synthesized sequence	YPZ3_0390

3. CONTENT

AmpliSens® *Yersinia pestis*-FRT PCR kit is produced in 1 form:

variant FRT REF R-B79(RG,iQ,Dt)-CE.

Variant FRT includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT *Yersinia pestis*	clear liquid from colorless to light lilac colour	0.6	1 tube
RT-PCR-mix-2-FEP/FRT	colourless clear liquid	0.3	1 tube
Polymerase (TaqF)	colourless clear liquid	0.03	1 tube
Positive Control DNA *Yersinia pestis* / STI (C+$_{Y.pestis\ /\ STI}$)	colourless clear liquid	0.1	1 tube
DNA-buffer	colourless clear liquid	0.6	1 tube
Internal Control STI-87 (IC)*	colourless clear liquid	0.6	1 tube

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

Variant FRT is intended for 60 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- 0.15 M NaCl (saline solution) or potassium-phosphate buffer (PBS) (sodium chloride, 137 mM; potassium chloride, 2.7 mM; sodium monophosphate, 10 mM; potassium diphosphate, 2 mM; pH 7.5±0.2) for pretreatment of autopsy material or in case of viral cultures testing.
- Glycerol 100% (in case of storage of human and animal faeces)
- Reagent for pre-treatment of sputum.
- Transport medium for storage and transportation of respiratory swabs.
- Homogeniser TissueLyser LT (Qiagen, Germany) recommended for homogenisation of organ atissues, ticks and fleas. Stainless steel beads with the diameter of 5 mm and 7 mm.
- 0.1 % sodium merthiolate solution. To prepare the solution, dissolve 0.1 g of merthiolate in 100 ml of sterile 0.9 % saline solution. The obtained 0.1 % methiolate solution is to be stored in a flask made of dark glass for no longer than 3 months at a temperature range from 2 to 8 °C.
- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette filter tips (up to 100 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Water bath with the temperature range from 25 to 100 °C, suitable for Eppendorf tubes.
- Microcentrifuge up to 16,000 g, suitable for Eppendorf tubes (for example, Hettish, Germany)
- Vacuum aspirator with a trap flask.
- Real-time instruments (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (Qiagen, Germany); iCycler iQ5 (Bio-Rad, USA), or equivalent).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
  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 manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Yersinia pestis*-FRT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the biological material.

The following material is used for analysis:

- Fleas;
- Ticks;
- Bird pellets;
- Soil;
- Human biological material:
  - — Whole blood;
  - — Bubo aspirate (vesicle, pustule, carbuncle) is obtained using a syringe of no less than 5 ml. First, cleanse the skin area to be punctured with 70% ethanol, then dub with a tincture of iodine (5 %), and once again with 70 % ethanol. Insert the needle so that its tip reaches the central part of the bubo, then pull the hub as much as possible and slowly eject the needle. Since the exudation of the pestilential bubo is located between the solid tissues, the volume of exudation withdrawn with the syringe is usually negligible and often fills only the lumen of the needle. After removing the needle from the bubo, collect 0.5 ml of 15 mM saline solution with it and empty the contents into an Eppendorf tube. If it is impossible to obtain a sample of material, inject 0.3–0.5 ml of sterile isotonic saline solution into the bubo. If the bubo bursts, collect the material separately from the solid peripheral area and from the fistula discharge. Analyse both portions separately.
  - — Sputum;
  - — In plague patients with localisation of primary pestilential buboes in the head and neck area, the oropharyngeal swabs are additionally collected with a sterile probe;
  - — Urine.
- Animal material
  - — Blood;
  - — Lymph nodes; liver, spleen, lung, adrenal gland, and brain tissues; and pathologically modified tissues and organs;
  - — Faeces.

The biological material should be delivered to the laboratory in the ice container within one day.

The samples are to be stored at 2–8 °C for 1 day or at the temperature not more than minus16 °C for 6 months.

Further analysis is performed according to the **DNA-sorb-B** REF K1-2-50-CE protocol.

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

Pretreatment of material:

1. **Fleas**
   One test sample may contain up to 30 fleas. Insects should be ground in a homogenizer or in a sterile mortar with a sterile pestle. Add 0.5 ml of a sterile 0.9 % saline solution or PBS and mix thoroughly. Centrifuge the suspension at 500 g for 2 min (3,000 rpm with 50 mm radius rotor), then transfer **100 µl of the top phase** into 1.5-ml tubes and use at the disinfection stage.
2. **Ticks**
   A pooled sample of imagoes may contain up to 3 blood-filled individuals, up to 10 hungry individuals; a pooled sample of nymphs: up to 10 blood-filled individuals and up to 30 hungry individuals; and a pooled sample of larvae: up to 30 blood-filled individuals. Pierce the blood-filled imagoes with a needle to release the blood prior to homogenisation in mortar. When using a closed-type homogenizer, this procedure can be omitted. Grind the sample using a homogenizer or a sterile mortar and pestle. Add 1 ml of a sterile 0.9 % saline solution or PBS and mix thoroughly. Centrifuge the suspension at 500 g for 2 min (3,000 rpm with 50 mm radius rotor) and transfer **50 µl of the top phase** to 1.5-ml tubes for DNA extraction.
3. **Human and animal faeces**
   Preparation of 10–20 % suspension:
   — Add 2 ml of saline solution or PBS to 5-ml tubes with tightly closable caps.
   — Add 0.5–1.0 g (~ 0.5–1.0 ml) of faeces to each tube using the single filter tips (or a disposable scapula). Mix thoroughly to obtain a homogenous suspension. If it is necessary to store the samples, add glycerol to the suspension (final concentration, 20 %), mix it, and store at ≤ –16 °C.
   — Preparation of faecal bacterial fraction:
   Transfer 1 ml of the suspension into 1.5-ml tubes with tightly closable caps and centrifuge at 8,000 g for 5 min. Use **100 µl of the clarified fraction** taken from the boundary of the transparent liquid fraction and solid faecal fractions to extract DNA.
4. **Bird pellets**
   Thoroughly grind bone marrow fragments from bone remains in bird pellets using a homogenizer or a sterile mortar and pestle. Add sterile 0.9 % saline solution or PBS (at least 500 µl) to obtain a 10 % suspension and mix thoroughly. Allow the suspension to settle at room temperature for 2–3 min and then transfer the top phase into 1.5-ml tubes. Use **100 µl of the suspension** to extract DNA.
5. **Soil**
   Transfer 0.4–1.0 g (~ 1.0 ml) of soil into 5-ml tubes with tightly closable caps using disposable spatula. Add 3 ml of 0.9 % saline solution, mix thoroughly, and allow to settle for 5 min. Transfer 1 ml of solution from tubes with settled ground into 1.5-ml tubes with tightly closable cap. Precipitate the coarsely dispersed fraction by centrifuging at 300 g for 2–3 min (2300 rpm, 50 mm radius rotor). Use **100 µl of the clarified supernatant** to extract DNA.
6. **Blood**
   Collect the whole blood specimen in the morning on an empty stomach to a tube with 6 % EDTA (ratio, 1:20). Invert the closed tube several times. Transfer 1.5 ml of the whole blood with EDTA into an Eppendorf tube. Centrifuge at 380 g for 10 min (800 rpm with 50 mm radius rotor). Transfer the top layer of plasma with leucocytes (500–600 µl) into another Eppendorf tube and centrifuge it at 8,000 g for 5 min. Transfer the supernatant (leaving ~200 µl of liquid above the cell pellet) into a container with a disinfectant. Use the **cell pellet and 200 µl of the supernatant** to extract DNA.
   If blood clots from the heart and large vessels of animals are used for analysis, the procedure of sampling is the same as for organs.
7. **Sputum**
   Pre-treatment is performed in accordance with the instruction manual for the **Mucolys**in reagent REF 180-CE . Use **50 µl of the sample** for DNA extraction.
8. **Oropharyngeal swabs**
   Oropharyngeal swabs are collected using sterile dry probes with cotton tips from the surface of tonsils, palatine arches, and the posterior oropharynx. After sampling, transfer the part of the probe with the cotton swab to a sterile disposable tube containing 500 µl of the **Transport medium for storage and transportation of respiratory swabs** REF 957-CE (or sterile saline or PBS). Break the end of probe or cut it with sterile scissors so that the cap of the tube can be sealed tightly. Close the tube with the solution and the probe fragment. Before starting work, the probe should be removed from the tube and discarded into disinfectant. Use **100 µl of the sample** to extract DNA.
9. **Urine**
   Collect urine into a clean container. When storage is required, transfer the urine sample into a 20-ml centrifuge tube or an Eppendorf tube, add glycerol (10 % of the sample volume), and mix until it evenly spreads through the sample. Urine samples with glycerol can be stored at the temperature not more than minus 20 °C for 1 week or at the temperature not more than minus 70 °C for a long time.
   — When using a refrigerated centrifuge for 20-ml tubes (4 °C, 8,000 g) the following pretreatment procedure is used:
   Centrifuge the sample at 8,000–9,000 g for 10 min. Discard the supernatant (except for 1 ml of liquid above the cell pellet) into a container with disinfectant. Transfer the cell pellet and 1 ml of the supernatant to an Eppendorf tube. Centrifuge the sample at 8,000 g for 10 min once again. Discard the supernatant (900 µl) into a container with disinfectant. Use the **pellet and 100 µl of the supernatant** to extract DNA. If the urine sample contains excess salts, only the **supernatant (100 µl)** is transferred into an Eppendorf tube and used to extract DNA.
   — In the absence of a centrifuge for 20-ml tubes with a speed of 8,000 g, concentrate bacteria from only 1 ml of urine as specified above. Use the **pellet and 100 µl of the supernatant** to extract DNA.
10. **Organs**
    Thoroughly grind the organ fragments (no less than 0.5 cm^3 in size) and lymph nodes (whole) in a homogenizer or in a sterile mortar with pestle. Add sterile 0.9 % saline solution (no less than 500 µl) or PBS and mix thoroughly. Allow the suspension to settle at room temperature for 2–3 min and then transfer the top phase into 1.5-ml tubes. Use **50 µl of the suspension** to extract DNA.

Before disinfection and DNA extraction, the pretreated material can be stored at no higher than minus 20 °C for 1 month or at the temperature not more than minus minus 70 °C for a long time.

7. WORKING CONDITIONS

AmpliSens® *Yersinia pestis*-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:

- **RIBO-prep**, REF K2-9-Et-50-CE.

Extract DNA from all types of biological material as described in the instruction manual to the RIBO-prep reagent kit with some modifications specified below:

1. Prior to DNA extraction, test material must be disinfected.
2. Add **10 µl** of **Internal Control STI-87 (IC)** and **300 µl** of **Solution for Lysis** to the tube labelled **C–** (Negative Control of Extraction).

NOTE:

3. After adding **Internal Control STI-87 (IC)** and heating at 65 °C for 5 min, centrifuge the tubes at 5,000 rpm for 5 s to be sure there are no drops on the caps.
4. Centrifuge the tubes at 13,000 rpm for 2 min:
   - after adding **500 µl** of **Washing Solution 3**;
   - after adding **200 µl** of **Washing Solution 4**.

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

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

1. Prepare the required number of tubes. Take into account that each run includes at least three controls: Negative Control of Extraction (C–) and Positive and Negative Controls of Amplification ($C+_{Y.pestis\ /\ STI}$ and NCA). Take excess reagents including one extra reaction in your calculation.
2. Mix in a new tube **PCR-mix-1-FRT *Yersinia pestis***, **RT-PCR-mix-2 FEP/FRT** and **polymerase (TaqF)**, calculating per each reaction:
   — **10 µl PCR-mix-1-FRT *Yersinia pestis*;**
   — **5 µl RT-PCR-mix-2-FEP/FRT;**
   — **0.5 µl polymerase (TaqF).**
3. Prepare the required number of tubes for DNA amplification from test and control DNA samples.
4. Add **15 µl** of the prepared mixture into each tube.

NOTE: Do not store the prepared mixture.

5. Using filter tips , add **10 µl** of **DNA samples** obtained from test or control samples at the DNA extraction stage into the tubes with the reaction mixture. Carefully mix the contents of the tubes by pipetting.
6. Carry out the control amplification reactions:

NCA	–	Add **10 µl** of **DNA-buffer** to the tube labeled **NCA** (Negative Control of Amplification).
$C+_{Y.pestis\ /\ STI}$	–	Add **10 µl** of **Positive Control DNA *Yersinia pestis* / STI** to the tube labeled **C+**.
C–	–	Add **10 µl** of **the sample extracted from the Negative Control of extraction** to the tube labeled C–.

8.2.2. Amplification

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

Table 2

Amplification program for Rotor-type instruments[1] and plate-type instruments[2]

Step	Temperature, °C	Time	Cycles
1	95	15 min	1
2	95	5 s	5
	60	20 s	
	72	15 s	
3	95	5 s	40
	60	30 s ***Fluorescent signal detection***	
	72	15 s	

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

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines [2].
3. Insert tubes into the reaction module of the instrument.

NOTE: Well № 1 must be filled with any test tube from the experiment, except for an empty one.

4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000, or equivalent.
[2] For example, iCycler iQ5, or equivalent.

9. DATA ANALYSIS

The results are interpreted by the software of the real-time PCR instrument used by measuring the fluorescence signal accumulation in two channels:

– The signal of the Internal Control STI-87 DNA amplification product is detected in the channel for the FAM fluorophore;
– The signal of the *Yersinia pestis* DNA amplification product is detected in the channel for the JOE fluorophore.

The results are interpreted by the presence (or absence) of an intercept between the fluorescence curve and 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.

The principle of interpretation is the following:

— *Yersinia pestis* DNA is **detected** in a sample if its *Ct* value detected in the result grid in the channel for the JOE fluorophore is less than the boundary *Ct* value, specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of the sample should intercept the threshold line in the area of typical exponential growth of fluorescence.

— *Yersinia pestis* DNA is **not detected** in a sample if its *Ct* value detected in the result grid in the channel for the FAM fluorophore is less than the specified boundary *Ct* value, whereas the *Ct* value in the channel for the JOE fluorophore is not detected.

— The result is **invalid** if the *Ct* value of a sample in the channel for the JOE fluorophore is absent whereas the *Ct* value in in the channel for the FAM fluorophore is also absent or is greater than the specified boundary *Ct* value. In such cases, the PCR analysis should be repeated starting from the DNA extraction stage.

NOTE: The boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed in the PCR kit. See also the 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 3).

Table 3

Results for controls

Control	Stage for control	*Ct* value in the channel for the fluorophore	
		JOE	FAM
C–	DNA extraction	Absent	< boundary *Ct* value
NCA	PCR	Absent	Absent
C+$_{Y.pestis\ /\ STI}$	PCR	< boundary *Ct* value	< boundary *Ct* value

10. TROUBLESHOOTING

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

1. If the *Ct* value of the Positive Control of Amplification (C+$_{Y.pestis\ /\ STI}$) in the channel for the JOE fluorophore is absent or greater than the specified boundary *Ct* value, then the PCR analysis should be repeated for all samples where the *Yersinia pestis* DNA was not detected.
2. If the *Ct* value of the Negative Control of Extraction (C–) is detected in the channel for the JOE fluorophore, then the PCR analysis should be repeated for all samples where *Yersinia pestis* DNA was detected.
3. If the *Ct* value is present for the Negative Control of Amplification (NCA) in channels for the FAM and/or JOE fluorophores, then the PCR analysis should be repeated for all samples where *Yersinia pestis* DNA was detected, with simultaneous analysis of NCA in at least three repeats.

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

11. TRANSPORTATION

AmpliSens® *Yersinia pestis*-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® *Yersinia pestis*-FRT** PCR kit (except for PCR-mix-1-FRT *Yersinia pestis*, RT-PCR-mix-2-FEP/FRT, and polymerase (TaqF)) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Yersinia pestis*-FRT** PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

NOTE: PCR-mix-1-FRT *Yersinia pestis*, RT-PCR-mix-2-FEP/FRT, and polymerase (TaqF) are to be stored at the temperature range from minus 24 to minus 16 °C when not in use.

NOTE: PCR-mix-1-FRT *Yersinia pestis* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Yersinia pestis*-FRT** PCR kit is specified in the table below.

Biological material (volume of sample)	RNA/DNA extraction kit	PCR kit	Analytical sensitivity	Biological material pretreatment
- fleas (30 specimens homogenized in 500 µl of PBS, sample volume 100 µl); - *Dermacentor reticulatus* ticks (pool of 10 specimens, sample volume 50 µl); - blood (200 µl); - urine (100 µl); - sputum (50 µl); - faeces (100 µl of 10 % suspension); - 10 % suspension of liver tissue, lymph nodes (50 µl)	RIBO-prep	variant FRT	1 x 10^3 copies/ml	The claimed sensitivity is achieved only when the material pretreatment is carried out in accordance with chapter *Sampling and Handling* and the recommended volume of test sample is used

13.2. Specificity

The analytical specificity of **AmpliSens® AmpliSens® *Yersinia pestis*-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 analytical specificity of **AmpliSens® *Yersinia pestis*-FRT** PCR kit was assessed using the following microorganisms: *Yersinia enterocolitica* (326 strains), *Y.pseudotuberculosis* (145 strains); *Shigella sonne*, *Sh.flexneri*; *Salmonella typhi*, *S.enteritidis*; *Klebsiella pneumonia*; *Esherichia coli* NCTC 9001; *Enterococcus faecalis*; *Staphylococcus aureus*, *St.saprophyticus*; *Pseudomonas aeruginosa*; *Proteus mirabilis*; and *Enterobacter cloacae*.

No false-positive results were observed during analysis of DNA of the above-mentioned microorganisms as well as DNA of ticks, mosquitoes, birds, and humans.

The clinical specificity of **AmpliSens® *Yersinia pestis*-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 AmpliSens® *Yersinia pestis*-FRT PCR Kit for detection of *Yersinia pestis* DNA in human biological material (blood; bubo aspirate, vesicle aspirate, pustule aspirate, carbuncle aspirate; sputum; oropharyngeal swabs; urine; faeces; lymph nodes; liver, spleen, lungs, adrenal, and brain tissues; as well as pathologically modified tissues and organs), animal material (blood, faeces, parenchymal organs, brain tissues, and pathologically changed tissues and organs), fleas, ticks, bird pellets and soil using real-time fluorescence-hybridization detection.

15. QUALITY CONTROL

In accordance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *Yersinia pestis*-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
13.03.19 EM	3. Contents	The colour of the reagent was specified
20.05.20 VA	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
12.03.21 MM	—	The name, address and contact information for Authorized representative in the European Community was changed

AmpliSens®

EC REP

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