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For Professional Use Only

AmpliSens® *Bordetella* multi-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

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

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1. INTENDED USE

AmpliSens® *Bordetella* multi-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of genome specific fragments of pathogens causing pertussis (*Bordetella pertussis*), parapertussis (*Bordetella parapertussis*), and *Bordetella bronchiseptica* infection (*Bordetella bronchiseptica*) in clinical material (swabs taken from lower nasal passage and back of oropharynx as well as culture of microorganisms) by using real-time hybridization-fluorescence detection.

The results of PCR analysis are to be taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Detection of DNA of the claimed pathogens by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. In real-time PCR, the amplified product is detected using 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® *Bordetella* multi-FRT** PCR kit is a qualitative test that contains the Internal Control (IC STI-87). 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® *Bordetella* multi-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 a chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

During the amplification four following reactions are running in one tube:

- amplification of the conservative fragment of gene *ptxA* encoding pertussis toxin found in genomes of *Bordetella pertussis, Bordetella parapertussis*, and *Bordetella bronchiseptica,*
- identification of specific fragment of *Bordetella pertussis* genome,
- identification of specific fragment of *Bordetella bronchiseptica* genome,
- amplification of the internal control sample sequence.

Detection channel	FAM/Green	JOE/Yellow/HEX	ROX/Orange	Cy5/Red
Reaction	IC detection	Detection of pertussis toxin gene, *ptxA*	Identification of *Bordetella pertussis*	Identification of *Bordetella bronchiseptica*

If the pertussis toxin gene is found in a sample (JOE/Yellow/HEX channel) it means that of the microorganism belonging to *Bordetella* genus (*B.pertussis, B.parapertussis*, or *B.bronchiseptica*) is present.

If positive results are simultaneously obtained in both JOE/Yellow/HEX and ROX/Orange channels it means that *Bordetella pertussis* is present in a sample. If positive results are simultaneously obtained in both JOE/Yellow/HEX and Cy5/Red channels it means that *Bordetella bronchiseptica* is present in a sample.

Presence of *Bordetella parapertussis* in a sample can be concluded from the following: detection of pertussis toxin gene (JOE/Yellow/HEX channel) along with the negative results of identification of *Bordetella pertussis* and *Bordetella* bronchiseptica in case of sufficient amount of *Bordetella* DNA in a sample, that is determined by the boundary Ct values specified in the Guidelines [2].

3. CONTENT

AmpliSens® *Bordetella* multi-FRT PCR kit is produced in 1 form:

AmpliSens® *Bordetella* multi-FRT variant FRT-100 F (for use with RG, iQ)

REF R-B84-100-F(RG,iQ,Dt)-CE

AmpliSens® *Bordetella* multi-FRT PCR kit variant FRT-100 F includes:

Reagent	***Description***	***Volume, ml***	***Quantity***
PCR-mix-1-FL-F *Bordetella* multi	colorless clear liquid	0.2	5 tubes
PCR-mix-2-FRT	colorless clear liquid	0.6	1 tube
Polymerase (TaqF)	colorless clear liquid	0.06	1 tube
Positive Control DNA *Bordetella* spp. (C+*Bordetella* spp.)	colorless clear liquid	0.1	2 tubes
Positive Control STI-88 (CS+)	colorless clear liquid	0.1	2 tubes
TE-buffer	colorless clear liquid	0.5	2 tubes
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Internal Control STI-87 (IC)**	colorless clear liquid	0.6	2 tubes

* 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.

AmpliSens® *Bordetella* multi-FRT PCR kit is intended for 100 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- Transport medium for storage and transportation of respiratory swabs (a reagent for storage of nasal and oropharyngeal swabs).
- 0.9% saline solution or 0.01 M potassium-phosphate buffer, pH 7.0 (if cultures of microorganisms are studied).
- Ultra minitip flocked swab, nylon tip, plastic applicator, sterile (516CS01, COPAN, Italy) – a probe for collecting lower nasal passage swabs in children.
- Flexible nasopharyngeal flocked swab, nylon tip, plastic applicator, sterile (503CS01, COPAN, Italy) – a probe for collecting lower nasal passage swabs in adults.
- Sterile swab, polystyrene+viscose, individually wrapped (300202, Deltalab, Spain) – a probe for collecting oropharyngeal swabs in children and adults
- DNA extraction kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Personal PCR cyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (Qiagen, Germany); iCycler iQ, iQ5 (Bio-Rad, USA)).
- Disposable polypropylene tubes for PCR of 0.2- or 0.1-ml:
    a) 0.2 ml domed cap PCR tubes (for example, Axygen, USA) if plate-type instrument is used;
    b) 0.2 ml flat cap PCR tubes (for example, Axygen, USA) or strip tubes 0.1 ml (4 tubes) (for example, Corbett Research, Australia or Qiagen, Germany) if rotor-type instrument is used;
- Refrigerator for 2–8 °C.
- Deep-freezer at ≤ –16 °C.
- Reservoir 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 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 protective gloves and laboratory cloths, and protect eye 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 compliance with appropriate biosafety practices.
- Clean and disinfect all specimens or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid specimens and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact,, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) 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 the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® ***Bordetella*** **multi-FRT** PCR kit is intended to analyze DNA extracted with DNA extraction kits from swabs taken from lower nasal passage, back of the oropharynx, and cultures of microorganisms.

Sampling

- *Lower nasal passage swabs* are taken with a nasopharyngeal nylon probe with a

plastic applicator. Prior sampling make a patient blow his nose if his nasal cavity is filled with mucous. Insert the probe gently along the external nasal wall to a depth of 2–3 cm towards the inferior nasal concha. Then move the probe slightly lower, insert it in the inferior nasal meatus under the inferior nasal concha, rotate, and remove along the external nasal wall.

When the material is obtained, place the working part of the probe with the cotton swab in a sterile disposable tube with 500 µl of transport medium for storage and transportation of respiratory swabs. Break off the terminal part of the probe to allow tight closing of the tube cup. Close the tube with the solution and the working part of the probe, and mark it.

- *Oropharyngeal swabs* are taken with a probe with a dry viscose swab. Take swabs by rotating the probe over the surface of tonsils, palatine arches, and the posterior wall of the pharynx.

  When the material is obtained, place the working part of the probe with the viscose swab in a sterile disposable tube with 500 µl of transport medium for storage and transportation of respiratory swabs. Break off the terminal part of the probe to allow tight closing of the tube cap. Close the tube with the solution and the working part of the probe, and mark it.

Storage of clinical specimens is allowed at 2–8 °C for 3 days or at ≤ –16 °C for 1 week.

It is recommended to combine nasopharyngeal and oropharyngeal swabs. To do this, working ends of probes are placed in one tube with 500 µl of medium for storage and transportation of respiratory swabs and studied as one sample.

- *Microorganism cultures* are resuspended in 1 ml of 0.9 % saline or 0.01 M potassium-phosphate buffer, pH 7.0. The obtained suspension is used for subsequent DNA extraction.

Pretreatment

Lower nasal passage swabs and *oropharyngeal swabs:* vortex the tube then centrifuge at 5,000 g for 5 s to remove drops from the tube cap.

7. WORKING CONDITIONS

AmpliSens® *Bordetella* multi-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-sorb, REF K2-1-Et-100-CE.
- RIBO-prep, REF K2-9-Et-100-CE.
- NucliSENS easyMAG (bioMérieux, France) automated system can also be used.

DNA extraction from every clinical sample is carried out in presence of **IC STI-87**. As negative control of extraction, Negative Control (C–) is to be used.

8.1.1 RNA extraction with the use of RIBO-sorb reagent kit:

Extract RNA according to the manufacturer's instruction.

- Volume of the Internal Control STI-87 reagent added per each tube is10 µl;

- Volume of the Negative Control reagent added into the tube for Negative Control of Extraction (C–) is100 µl;
- Volume of a clinical sample is 100 µl;

- If suspended particles are seen in the tubes after adding clinical samples (undissolved clinical material), centrifuge the tubes at 10,000 rpm for 1 min and transfer supernatant to new tubes

- Purified DNA can be stored at 2–8 °C for 1 week, at ≤–16 °C for 1 year, at ≤–68 °C for a long time

8.1.2 RNA extraction with the use of RIBO-prep reagent kit:

Extract RNA according to the manufacturer's instruction.

- Volume of the Internal Control STI-87 reagent added per each tube is10 µl;

- Volume of the Negative Control reagent added into the tube for Negative Control of Extraction (C–) is 100 µl;
- Volume of clinical sample is 100 µl;

- Purified DNA can be stored at 2–8 °C for 1 week, at ≤–16 °C for 1 year, at ≤–68 °C for a long time

8.1.3 RNA extraction with the use of NucliSENS easyMAG automated system

DNA extraction with NucliSENS easyMAG automated system is described in Guidelines [2].

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

1. Prepare the required number of tubes with **PCR-mix-1-FL-F *Bordetella* multi** for amplification of DNA from clinical and control samples. Thaw the tubes with **PCR-**

mix-1-FL-F *Bordetella* multi. Vortex the tubes with **PCR-mix-1-FL-F *Bordetella* multi**, **PCR-mix-2-FRT**, and **polymerase (TaqF)** and then centrifuge briefly (1-2 s).

2. Prepare the required number of tubes or strips (including controls) for DNA amplification.
3. For N reactions, add to a new tube:
   - **10*(N+1) µl** of **PCR-mix-1-FL-F *Bordetella* multi**,
   - **5.0*(N+1) µl** of **PCR-mix-2-FRT**,
   - **0.5*(N+1) µl** of **polymerase (TaqF)**.
4. Vortex the tube with the prepared mixture and then centrifuge it briefly.
5. Transfer **15 µl** of the prepared mixture to the prepared tubes.
6. Add **10 µl** of **DNA** obtained after nucleic acid extraction from clinical or control samples.
7. Carry out the control reactions:

NCA - Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative control of amplification).

C+ - Add **10 µl** of **Positive Control DNA *Bordetella* spp.** to the tube labeled C+ (Positive control of amplification).

CS+ - Add **10 µl** of **Positive Control STI-88** to the tube labeled CS+ (Positive control of IC amplification).

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

It is recommended to sediment drops from walls of tubes by short vortex (1–3 s) before their insertion in the thermocycler.

8.2.2. Amplification

1. Program the thermocycler according to **Manufacturer's manual** and **Guidelines [2]**.
2. Create a temperature profile on your instrument as follows:

Table 1

***Bordetella* multi-FRT amplification program**

	***Rotor-type instruments*[1]**			***Plate-type instruments*[2]**		
Step	**Temperature, °C**	**Time**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
1	**95**	**15 min**	1	**95**	**15 min**	1
2	**95**	10 s	10	**95**	10 s	10
	60	20 s		**60**	25 s	
	72	10 s		**72**	25 s	
3	**95**	10 s	35	**95**	10 s	35
	60	20 s Fluorescence detection		**60**	25 s Fluorescence detection	
	72	10 s		**72**	25 s	

Fluorescent signal is detected in the FAM/Green, JOE/Yellow/HEX, ROX/Orange, and Cy5/Red channels.

3. Place PCR tubes into the PCR instrument.
4. Run amplification and signal detection program.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

The results are interpreted by the software of the used real-time PCR instrument 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 the *Ct* (cycle threshold) value in the results grid.

Principle of amplification data analysis:

Curves of fluorescence signal accumulation are analyzed in four channels:

Detection channel	**FAM/Green**	**JOE/Yellow/HEX**	**ROX/Orange**	**Cy5/Red**
Reaction	IC detection	Detection of pertussis toxin gene, *ptxA*	Identification of *Bordetella pertussis*	Identification of *Bordetella bronchiseptica*

– the amplification product of the pertussis toxin gene fragment which is found in the genomes of *Bordetella pertussis, Bordetella parapertussis,* and *Bordetella bronchiseptica* is detected in the JOE/Yellow/HEX channel,

– the amplification product of the specific fragment of the *Bordetella pertussis* genome is detected in the ROX/Orange channel,

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q.
[2] For example, iCycler iQ, iQ5.

– the amplification product of the specific fragment of the *Bordetella bronchiseptica* genome is detected in the Cy5/Red channel,

– the amplification product of the Internal Control STI-87 (IC) DNA is detected in the FAM/Green channel.

The result of amplification is considered to be **positive** if the fluorescent curve have a typical S-shape, crosses the threshold line in the area of reliable growth of fluorescence, and *Ct* value in the results grid for a particular channel is less than the specified boundary Ct value. The result of amplification is considered to be **negative** if the fluorescence curve does not have a typical S-shape, does not cross the threshold line (no *Ct* value), or if obtained *Ct* value exceeds the specified boundary Ct value.

Ct (cycle threshold) values are indicated in the *Important Product Information Bulletin* enclosed in the PCR kit. See also the Guidelines [2]

The results of analysis are considered reliable only if the results obtained for positive and negative controls of amplification and negative control of extraction are correct (see Table 2).

Table 2

Results for controls

Control	Stage for control	***Ct* value**			
		FAM/Green	**JOE/Yellow/HEX**	**ROX/Orange**	**Cy5/Red**
		Detection of IC	**Detection of pertussis toxin gene**	**Identification of *Bordetella pertussis***	**Identification of *Bordetella bronchiseptica***
C–	DNA extraction	< boundary Ct value*	absent	absent	absent
NCA	PCR	absent	absent	absent	absent
CS+	PCR	< boundary Ct value*	absent	absent	absent
C+	PCR	absent	< boundary Ct value*	< boundary Ct value*	< boundary Ct value*

* For boundary Ct values, see the *Important Product Information Bulletin* enclosed in the PCR kit and the Guidelines [2].

Principle of interpretation of results:

Results of PCR analysis for detection and differentiation of pathogens that cause pertussis (*Bordetella pertussis*), parapertussis (*Bordetella parapertussis*), and *Bordetella bronchiseptica* infection (*Bordetella bronchiseptica*) are based on combinations of amplification results interpreted in accordance with the Table 3.

Table 3

Interpretation of results of PCR-analysis for clinical samples

Variants	FAM/Green	JOE/Yellow/HEX	ROX/Orange	Cy5/Red	Interpretation
	***Ct* value**				
1	absent or > boundary Ct value*	absent or present but > boundary Ct value*	absent	absent	**Invalid**
2	< boundary Ct value*	absent	absent	absent	*B.pertussis* *B.parapertussis* *B.bronchiseptica* **NOT detected**
3	present or absent	present	present	absent	*B.pertussis* DNA **detected**
4	present or absent	present	absent	present	*B.bronchiseptica* DNA **detected**
5	present or absent	present and < boundary value*	absent	absent	*B.parapertussis* DNA **detected**
6	< boundary Ct value*	present but > boundary value*	absent	absent	*Bordetella* spp. DNA **detected**: *B.pertussis,* or *B.parapertussis,* or *B.bronchiseptica.* For species differentiation, repeat sampling
7	< boundary Ct value*	absent	present	absent	If the result is repeated in PCR, interpret as **equivocal**
8	< boundary Ct value*	absent	absent	present	If the result is repeated in PCR, interpret as **equivocal**

* For boundary Ct values, see the *Important Product Information Bulletin* enclosed in the PCR kit and the Guidelines [2].

- **DNA of *B.pertussis, B.parapertussis* and *B.bronchiseptica*** are **not detected** in a sample if *Ct* value in the JOE/Yellow/HEX, ROX/Orange, and Cy5/Red channel is not determined (fluorescence curve does not cross the threshold line), whereas *Ct* value determined in the FAM/Green channel (detection of the Internal Control) does not exceed the specified boundary Ct value.
- **DNA of *B.pertussis*** is **detected** in a sample if *Ct* value detected in the JOE/Yellow/HEX and ROX/Orange channels does not exceed the specified boundary Ct value. Moreover, the fluorescence curve of this sample should have typical exponential growth. For such samples *Ct* detected in the FAM/Green channel (detection of the Internal Control) can have any value or can be absent in case of high pathogen load.

- **DNA of *B.bronchiseptica*** is **detected** in a sample if *Ct* value detected in the JOE/Yellow/HEX and Cy5/Red channels does not exceed the specified boundary Ct value. Moreover, the fluorescence curve of this sample should have typical exponential growth. For such samples *Ct* detected in the FAM/Green channel (detection of the Internal Control) can have any value or can be absent in case of high pathogen load.
- **DNA of *B. parapertussis*** is **detected** in a sample if *Ct* value determined in the JOE/Yellow/HEX channel is less than the boundary Ct value, whereas *Ct* value in the ROX/Orange and Cy5/Red channels is not determined (absent). Moreover, the fluorescence curve of this sample should have typical exponential growth. For such samples *Ct* determined in the FAM/Green channel (detection of the Internal Control) can have any value or can be absent in case of high pathogen load.
- If *Ct* value of a sample determined in JOE/Yellow/HEX channel is greater than the boundary Ct value, whereas Ct values are absent in the ROX/Orange and Cy5/Red channels and *Ct* value in the FAM/Green channel (detection of the Internal Control) is less than the boundary Ct value, it indicates that **DNA of one of the pathogens belonging to *Bordetella* genus is detected (*B.pertussis, B.parapertussis,* and *B.bronchiseptica*)**. However, quantity of the extracted DNA is not enough to discriminate the species, so for this purpose sampling of the clinical material should be repeated.
- If *Ct* value of a sample is absent in the result grid in the JOE/Yellow/HEX channel, but *Ct* values are determined in the ROX/Orange or Cy5/Red channels while *Ct* value in the FAM/Green channel (detection of IC) is less than the specified boundary Ct value, repeat PCR run. If the same result is obtained in the second run, the result of the sample is considered **equivocal** and sampling of clinical material should be repeated.
- The result of the analysis is considered **invalid**, if *Ct* value is not determined (absent) in the ROX/Orange and Cy5/Red channels, *Ct* value in the JOE/Yellow/HEX channel is absent or greater than the specified boundary Ct value and *Ct* value in the FAM/Green channel (detection of the Internal Control) is also absent or greater than the specified boundary *Ct* value. PCR analysis if this sample should be repeated beginning with DNA extraction stage.

10. TROUBLESHOOTING

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

1. If *Ct* value of the Positive Control of PCR (C+) is absent in the corresponding channel or greater than the boundary Ct value, amplification should be repeated for all negative clinical samples.
2. If *Ct* value is detected for the Negative Control of extraction (C–) in the JOE/Yellow/HEX, ROX/Orange, or Cy5/Red channels and/or *Ct* value is detected for the Negative Control of amplification (NCA) in any channel, PCR analysis should be repeated for all positive samples beginning with DNA extraction and take measures to detect and eliminate the source of contamination.

11. TRANSPORTATION

AmpliSens® *Bordetella* multi-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® *Bordetella* multi-FRT** PCR kit (except for PCR-mix-1-FL-F *Bordetella* multi, PCR-mix-2-FRT, and polymerase (TaqF)) are to be stored at 2–8 °C when not in use. All components of the AmpliSens® *Bordetella* multi-FRT PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FL-F *Bordetella* multi, 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-F *Bordetella* multi is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity for lower nasal passage swabs and oropharyngeal swabs

Pathogen	DNA/RNA extraction kit	Analytical sensitivity, GE/ml[3]
Bordetella pertussis **(pertussis pathogen)**	RIBO-sorb	$1x10^3$
	RIBO-prep	$5x10^2$
	NucliSENS easyMAG	$5x10^2$
Bordetella parapertussis **(parapertussis pathogen)**	RIBO-sorb	$1x10^3$
	RIBO-prep	$5x10^2$
	NucliSENS easyMAG	$5x10^2$
Bordetella bronchiseptica	RIBO-sorb	$1x10^3$

[3] Sensitivity is present in genomic equivalents (GE) of causative agent per 1 ml of sample.

	RIBO-prep	$5x10^2$
	NucliSENS easyMAG	$5x10^2$

13.2. Specificity

The analytical specificity of **AmpliSens® *Bordetella* multi-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.

AmpliSens® *Bordetella* multi-FRT PCR kit makes it possible to detect DNA of the specific fragments of the claimed pathogens. The specificity of this kit was confirmed by investigation of the following reference strains: *Streptococcus* spp*., Moraxella catarrhalis, Staphilococcus aureus, Staphilococcus saprophiticus, Haemophilus influenzae, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Mycobacteria tuberculosis* 27294 105*, Neisseria flava, Neisseria sicca, Neisseria mucosa, E. coli ATCC, NCTC,* 01577 27u7*, Enterococcus faecalis, Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila, Shigella flexneri, Shigella sonnei, Salmonella Enteritidis, Yersinia enterocollitica*, as well as human genomic DNA.

The clinical specificity of **AmpliSens® *Bordetella* multi-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, 2008.
2. Guidelines to AmpliSens® *Bordetella* multi-FRT PCR kit for qualitative detection and differentiation of genome specific fragments of pathogens causing pertussis (*Bordetella pertussis*), parapertussis (*Bordetella parapertussis*), and *Bordetella bronchiseptica* infection (*Bordetella bronchiseptica*) in clinical by using real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

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® *Bordetella* multi-FRT** 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
RUO	Research use only		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
RG	For working with Rotor-Gene 3000/6000 (Corbett Research)	**C+, CS+**	Positive control of amplification
iQ	For working with iQ5, iCycler iQ (Bio-Rad)	**IC**	Internal control