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For Professional Use Only

AmpliSens® *Bordetella* multi-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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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 the mucous membrane of inferior nasal meatus and posterior pharyngeal wall, cultures 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 (**Internal Control STI-87 (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® *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.

Channel for fluorophore	FAM	JOE	ROX	Cy5
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 (the channel for JOE fluorophore) 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 the channels for both JOE and ROX fluorophores it means that *Bordetella pertussis* is present in a sample. If positive results are simultaneously obtained in the channels for both JOE and Cy5 fluorophores 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 (the channel for JOE fluorophore) 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] and in the *Important Product Information Bulletin*

3. CONTENT

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

AmpliSens® *Bordetella* multi-FRT variant FRT-100 F **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	clear liquid from colorless to light lilac colour	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 STI-87 (IC)** 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.
- 0.9 % saline solution or 0.01 M potassium-phosphate buffer (pH 7.0) in case of microorganisms' cultures testing.
- Flocked or fiber swabs for collecting specimens from kids and adults.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free 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 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); iCycler iQ or iCycler iQ5 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml) when working with PCR kit variant FRT-100 F:
  a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  b) 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers 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 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 analysis of DNA extracted with DNA extraction kits from swabs taken from the clinical material (swabs taken from the mucous membrane of inferior nasal meatus and posterior pharyngeal wall, cultures of microorganisms).

Sampling

- *The swabs from the mucous membrane of inferior nasal meatus.*

  The material is taken using a sterile nasopharyngeal flocked swab with a plastic applicator. Prior sampling make a patient blow his nose if his nasal cavity is filled with

mucous. Insert the flocked swab gently along the external dorsum of the nose to a depth of 2–3 cm towards the inferior nasal concha. Then move the swab slightly lower, insert it in the inferior nasal meatus under the inferior nasal concha, rotate, and take it out along the dorsum of the nose.

After sampling place the working part of the swab in a sterile disposable tube with 500 µl of transport medium for storage and transportation of respiratory swabs. Break off the lower part of the swab to close tight the tube cap. Close the tube with the medium and the working part of the swab and mark it.

- *The swabs from posterior pharyngeal wall*

  The material is taken using 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.

  After sampling place the working part of the swab in a sterile disposable tube with 500 µl of transport medium for storage and transportation of respiratory swabs. Break off the lower part of the swab to close tight the tube cap. Close the tube with the medium and the working part of the swab, 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, use different swabs for sampling and place their working parts in one tube with 500 µl of medium for storage and transportation of respiratory swabs. Study as one sample.

- *Microorganism cultures* should be resuspended in 1 ml of 0.9 % saline or 0.01 M potassium-phosphate buffer, pH 7.0. Use the obtained suspension subsequent DNA extraction.

Pretreatment

Swabs taken from the mucous membrane of inferior nasal meatus and posterior pharyngeal wall: vortex the closed tube and then centrifuge it 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 automated system (for details see Guidelines [2]).

DNA extraction of each test sample is carried out in the presence of **Internal Control STI-87 (IC)**.

In the extraction procedure it is necessary to carry out the control reaction as follows:

C– – Add **100 µl of Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction).

Extract the DNA according to the manufacturer's protocol.

In case of extracting with RIBO-sorb or RIBO-prep reagent kits, make sure that the volume of **Internal Control STI-87 (IC)** reagent added to each tube is **10 µl.**

In case of extracting with the RIBO-sorb reagent kit, make sure that there are not suspended particles in the tubes before adding the sorbent. Otherwise, centrifuge the tubes at 10,000 rpm for 1 min and then transfer the supernatant to new tubes.

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**.

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

1. Thaw the required number of tubes with **PCR-mix-1-FL-F *Bordetella* multi** for amplification of DNA from clinical and control samples. 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. Take the required number of tubes/strips for amplification of the DNA obtained from clinical and control samples. The type of tubes depends on the PCR instrument used for analysis.
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 each tube.
6. Add **10 µl** of **DNA** obtained at the DNA extraction stage.
7. Carry out the control amplification 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).

8.2.2. Amplification

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

Table 1

Amplification program for *Bordetella* multi-FRT

	***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 acquiring		**60**	25 s Fluorescence acquiring	
	72	10 s		**72**	25 s	

Fluorescent signal is detected in the channels for FAM, JOE, ROX and Cy5 fluorophores.

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

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

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

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in four channels:

[1] For example, Rotor-Gene 3000/Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany).
[2] For example, iCycler iQ, iQ5 (Bio-Rad, USA).

Channel for fluorophore	FAM	JOE	ROX	Cy5
Reaction	IC detection	Detection of pertussis toxin gene, *ptxA*	Identification of *Bordetella pertussis*	Identification of *Bordetella bronchiseptica*

– The signal of 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 channel for JOE fluorophore,
– The signal of the amplification product of the specific fragment of the *Bordetella pertussis* genome is detected in the channel for ROX fluorophore,
– The signal of the amplification product of the specific fragment of the *Bordetella bronchiseptica* genome is detected in the channel for Cy5 fluorophore,
– The signal of Internal Control STI-87 (IC) DNA amplification product is detected in the channel for FAM fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.

Principle of interpretation

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 2.

Table 2

Interpretation of results of PCR-analysis

Variants	*Ct* value in the channel for fluorophore				Interpretation
	FAM	JOE	ROX	Cy5	
1	Absent or > boundary value	Absent or > boundary value	Absent	Absent	**Invalid result**
2	< boundary value	Absent	Absent	Absent	*B.pertussis* *B.parapertussis* *B.bronchiseptica* **are NOT detected**
3	Present or Absent	Present	Present	Absent	*B.pertussis* DNA **is detected**
4	Present or Absent	Present	Absent	Present	*B.bronchiseptica* DNA **is detected**
5	Present or Absent	< boundary value	Absent	Absent	*B.parapertussis* DNA **is detected**
6	< boundary value	> boundary value	Absent	Absent	*Bordetella* spp. DNA **is detected**: *B.pertussis,* or *B.parapertussis,* or *B.bronchiseptica.* For species differentiation, repeat sampling
7	< boundary value	Absent	Present	Absent	If the result of PCR is repeated, interpret as **equivocal**
8	< boundary value	Absent	Absent	Present	If the result of PCR is repeated, interpret as **equivocal**

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

- **DNA of *B.pertussis, B.parapertussis* and *B.bronchiseptica*** are **not detected** if the *Ct* value is not determined (absent) in the channels for JOE, ROX and Cy5 fluorophores (fluorescence curve does not cross the threshold line), whereas the *Ct* value determined in the channel for FAM fluorophore (detection of the Internal Control) does not exceed the boundary *Ct* value specified in the *Important Product Information Bulletin*.
- ***B.pertussis* DNA** is **detected** in a sample if the *Ct* value determined in the results grid in the channels for JOE and ROX fluorophores is less than the boundary *Ct* value specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of this sample should have typical exponential growth. For such samples the *Ct* value determined in the result grid in the channel for FAM fluorophore (detection of the

Internal Control) can have any value or is absent in case of high pathogen load.

- ***B.bronchiseptica*** **DNA** is **detected** in a sample if the *Ct* value determined in the results grid in the channels for JOE and Cy5 fluorophores is less than the boundary *Ct* value specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of this sample should have typical exponential growth. For such samples the *Ct* value determined in the result grid in the channel for FAM fluorophore (detection of the Internal Control) can have any value or is absent in case of high pathogen load.
- ***B. parapertussis*** **DNA** is **detected** in a sample if the *Ct* value determined in the results grid in the channel for JOE fluorophore is less than the boundary *Ct* value, whereas the *Ct* value in the channels for ROX and Cy5 fluorophores is not determined (absent). Moreover, the fluorescence curve of this sample should have typical exponential growth. For such samples the *Ct* value determined in the result grid in the channel for FAM fluorophore (detection of the Internal Control) can have any value or is absent in case of high pathogen load.
- If the *Ct* value determined in the results grid for a sample in the channel for JOE fluorophore is greater than the boundary *Ct* value, whereas *Ct* values in the channels for ROX and Cy5 fluorophores are absent and the *Ct* value in the channel for FAM fluorophore (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 the species identification, 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 channel for JOE fluorophore, whereas *Ct* values are determined in the channels for ROX or Cy5 fluorophores and the *Ct* value in the channel for FAM fluorophore (detection of IC) is less than the specified boundary *Ct* value, repeat PCR analysis for this sample. If the same result is repeated, the result is considered **equivocal** and sampling of clinical material should be repeated.
- The result is **invalid**, if *Ct* value is not determined (absent) in the channels for ROX and Cy5 fluorophores, the *Ct* value in the channel for JOE fluorophore is absent or greater than the specified boundary *Ct* value and the *Ct* value in the channel for FAM fluorophore (detection of the Internal Control) is also absent or greater than the specified boundary *Ct* value. Repeat PCR analysis for this sample beginning with DNA extraction stage.

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 fluorophore			
		FAM	**JOE**	**ROX**	**Cy5**
		Detection of IC	**Detection of pertussis toxin gene**	**Identification of *Bordetella pertussis***	**Identification of *Bordetella bronchiseptica***
C–	DNA extraction	**<**boundary value	Absent	Absent	Absent
NCA	PCR	Absent	Absent	Absent	Absent
CS+	PCR	**<**boundary value	Absent	Absent	Absent
C+	PCR	Absent	**<**boundary value	**<**boundary value	**<**boundary value

10. TROUBLESHOOTING

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

1. If the *Ct* value determined for the Positive Control of Amplification (C+) in the respective channel is greater than the boundary *Ct* value or absent, repeat the amplification for all negative clinical samples.
2. If *Ct* value is determined for the Negative Control of Extraction (C–) in the channel for JOE, ROX or Cy5 fluorophore and/or the *Ct* value is determined for the Negative Control of Amplification (NCA) in any channel, the PCR analysis should be repeated for all positive samples beginning with the DNA extraction and measures for detection and elimination the source of contamination should be taken.

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

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

PCR-mix-1-FL-F *Bordetella* multi, polymerase (TaqF), and PCR-mix-2-FRT are to be stored at the temperature from minus 24 to minus 16 °C.

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 **(*Bordetella bronchiseptica* infection pathogen)**	RIBO-sorb	$1x10^3$
	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.

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

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 **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 the clinical material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

15. QUALITY CONTROL

In 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
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+, CS+**	Positive control of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
09.04.15 PM	Through the text	Corrections according the template and Russian instruction manual
	8.1 DNA extraction	The chapter was rewritten. The control of extraction was described.
	9. Data analysis	The section was rewritten
26.12.17 PM	3. Content	The colour of the reagent was specified