



For Professional Use Only

AmpliSens[®] *Parvovirus* B19-FRT

PCR kit

Instruction Manual

AmpliSens[®]



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® Parvovirus B19-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of *Parvovirus* B19 DNA in the clinical material (peripheral and umbilical blood, plasma or serum of peripheral and umbilical blood, oropharyngeal washes and swabs, saliva, cerebrospinal fluid, bone marrow biopsy samples, amniotic fluid, chorionic villi, placental biopsy samples, transudate (ascitic fluid) in case of non-immune fetal hydrops) using real-time hybridization-fluorescence detection of amplified products.

This PCR kit can be used for screening donated blood as well as blood products.

AmpliSens® Parvovirus B19-FRT PCR kit was validated on the 2nd WHO International Standard for *Parvovirus* B19 DNA for Nucleic Acid Amplification (NAT) Assay (version 1.0, dated 04/02/2009, standard sample, NIBSC code 99/802).

Converting factor to change from *Parvovirus* B19 DNA copies/ml to IU/ml is 0.9 for **AmpliSens® Parvovirus B19-FRT** PCR kit.



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

2. PRINCIPLE OF PCR DETECTION

Parvovirus B19 detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using special *Parvovirus* B19 primers. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes that 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® Parvovirus B19-FRT PCR kit is a qualitative and quantitative 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® Parvovirus B19-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 using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

Detection of *Parvovirus* B19 DNA is based on:

- total DNA extraction from the clinical material along with the Internal Control (IC STI-87).
- simultaneous amplification (multiplex PCR) of the DNA fragment of the structural gene coding *Parvovirus* B19 VP1 protein and engineered DNA fragment cloned in Lambda phage DNA which is used as the exogenous noncompetitive internal control with hybridization-fluorescence detection.

A qualitative real-time PCR detection of *Parvovirus* B19 DNA is based on the linear subjection between the initial concentration of the DNA-target in the test sample and the cycle when the exponential growth of the fluorescent signal begins (Cycle threshold, *Ct*). To run a quantitative test, amplification of clinical samples is carried out simultaneously with the amplification of DNA calibrators (samples with the known concentration of the DNA target). Based on the amplification results of DNA calibrators a calibration line is plotted and it is used for the estimation of concentration of the DNA target in the examined samples.

3. CONTENT

AmpliSens® *Parvovirus* B19-FRT PCR kit is produced in 1 form:

AmpliSens® *Parvovirus* B19-FRT PCR kit variant FRT-50 F, **REF** R-V49(RG,iQ,Mx)-CE.

AmpliSens® *Parvovirus* B19-FRT PCR kit variant FRT-50 F includes:

<i>Reagent</i>		<i>Description</i>	<i>Volume, ml</i>	<i>Quantity</i>
PCR-mix-1-FRT <i>Parvovirus</i> B19		colorless clear liquid	0.6	1 tube
PCR-mix-2-FRT		colorless clear liquid	0.3	1 tube
Polymerase (TaqF)		colorless clear liquid	0.03	1 tube
DNA calibrators	KS1 B19	colorless clear liquid	0.2	1 tube
	KS2 B19	colorless clear liquid	0.2	1 tube
DNA-buffer		colorless clear liquid	0.5	1 tube
Positive Control DNA <i>Parvovirus</i> B19 / STI (C+B19/STI)		colorless clear liquid	0.1	1 tube
Negative Control (C-)*		colorless clear liquid	1.2	1 tube
Internal Control STI-87 (IC)**		colorless clear liquid	1.0	1 tube

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

** add 10 µl of Internal Control during the DNA extraction procedure directly to the

sample/lysis mixture (RIBO-prep, **REF** K2-9-Et-50-CE protocol.)

AmpliSens® Parvovirus B19-FRT PCR kit variant FRT-50 F is intended for 60 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit or the DNA extraction automatic station.
- 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 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany) iCycler iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA)).
- Disposable polypropylene 0.2 or 0.1-ml tubes:
 - a) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
 - b) thin-walled 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 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® Parvovirus B19-FRT PCR kit is intended for analysis of the DNA extracted with a DNA extraction kits from the clinical material (peripheral and umbilical blood, plasma or serum of peripheral and umbilical blood, oropharyngeal washes and swabs, saliva, cerebrospinal fluid, bone marrow biopsy samples, amniotic fluid, chorionic villi, placental biopsy samples, transudate (ascitic fluid) in case of non-immune fetal hydrops). This PCR kit can be used for screening donated blood as well as blood products.

6.1. *Peripheral or umbilical blood plasma.* Peripheral blood is taken in 3 hours after meals at least. Umbilical blood is taken during the cordocentesis in accordance with the standard procedure at 20-24 weeks of the pregnancy. Blood is collected into a tube with 6 % EDTA. The tube is closed and turned several times upside down to ensure proper mixing. The blood plasma is collected and transferred to a new tube within 6 h after the blood sampling and centrifuged at 800–1600 rpm for 10 min. Plasma samples are stored at 2–8 °C for 5 days at most, at ≤ –16 °C for 6 month at most, or

at ≤ -68 °C for long-term storage. Sample melting leads up to the degradation of the virus DNA so it is necessary to aliquot samples (0.2–0.5 ml) for long-term storage.

AmpliSens[®] *Parvovirus* B19-FRT PCR kit can be used for the analysis of individual and pooled samples. A minipool should consist of 10 individual samples at most (100 μ l of blood plasma obtained from each of 10 samples).

The DNA from 100 μ l of an individual blood plasma sample can be extracted with the use of the NucliSENS easyMAG automated system or RIBO-prep extraction kit. The DNA from a minipool can be extracted only with the use of the NucliSENS easyMAG automated system.

- 6.2. *Amniotic fluid* is obtained during the amniocentesis (aspiration method) in accordance with the standard procedure at 16–23 weeks of the pregnancy. A sample of at least 1.0-ml volume is collected in a sterile tightly sealing container.

The pretreatment of the sample is required. An amniotic fluid sample should be thoroughly resuspended. Remove 1.0 ml of the sample and transfer it into an Eppendorf tube using a pipette with a tip with an aerosol filter. Centrifuge the tube at 8,000–9,000 g (12,000-13,000 rpm) for 10 min. Carefully remove the supernatant using a tip with an aerosol filter leaving 200 μ l of the solution over the pellet. Resuspend the pellet by vortexing.

The amniotic fluid and pretreated material are stored at 2–8 °C for 1 day at most, at ≤ -16 °C for 1 month at most, or at ≤ -68 °C for long-term storage. Only one freeze–thaw cycle of clinical material is allowed.

- 6.3. *Saliva* sample (0.2–1.0 ml) is collected into a 1.5-ml sterile Eppendorf tube after the patient is rinsed his mouth with water three times before sampling. Saliva samples are stored at 2–8 °C for 1 day at most, at ≤ -16 °C for 1 month at most, or at ≤ -68 °C for long-term storage.

- 6.4. *Oropharyngeal swabs* are obtained using sterile dry rayon swabs with plastic shafts for oropharyngeal swabs. Rotate the swab over the surface of tonsils, palatine arches, and posterior wall of pharynx after gargling the oral cavity with water. When material is obtained, insert the swab into a sterile disposable tube with 500 μ l of transport medium. Break off the end of shaft to allow tight closing of tube cap. Close the tube with the solution and the swab. Swabs are stored at 2–8 °C for 1 day at most, at ≤ -16 °C for 1 month at most, or at ≤ -68 °C for long-term storage.

7. WORKING CONDITIONS

AmpliSens® *Parvovirus B19-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;
- NucliSENS easyMAG automated system (for details see Guidelines [2]).

The 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 reactions as follows:

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



Extract DNA according to the manufacturer's protocol.

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**.

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

1. All reaction components should be mixed just before analysis. Prepare the **reaction mixture** taking the following volumes for one reaction:
 - **10 µl of PCR-mix-1-FRT *Parvovirus B19*,**
 - **5.0 µl of PCR-mix-2-FRT,**
 - **0.5 µl of polymerase (TaqF).**

Make the calculation for the required number of reactions, including test and control samples according to table 1.

Reaction mixture preparation scheme

Volume of reagents is 15 µl				
Volume of reagent per one reaction, µl		10.0	5.0	0.5
Number of clinical samples		PCR-mix-1-FRT <i>Parvovirus</i> B19	PCR-mix-2-FRT	Polymerase (TaqF)
Quantitative detection ¹	Qualitative detection ²			
1	4	70	35	3.5
2	5	80	40	4.0
3	6	90	45	4.5
4	7	100	50	5.0
5	8	110	55	5.5
6	9	120	60	6.0
7	10	130	65	6.5
8	11	140	70	7.0
9	12	150	75	7.5
10	13	160	80	8.0
11	14	170	85	8.5
12	15	180	90	9.0
13	16	190	95	9.5
14	17	200	100	10.0
15	18	210	105	10.5
16	19	220	110	11.0
17	20	230	115	11.5
18	21	240	120	12.0
19	22	250	125	12.5
20	23	260	130	13.0
21	24	270	135	13.5
22	25	280	140	14.0
23	26	290	145	14.5
24	27	300	150	15.0
25	28	310	155	15.5
26	29	320	160	16.0
27	30	330	165	16.5
28	31	340	170	17.0
29	32	350	175	17.5
30	33	360	180	18.0

For qualitative detection it is necessary to carry out 2 controls (Positive Control DNA *Parvovirus* B19 / STI and DNA-buffer),

For quantitative detection it is necessary to carry out 5 controls (2 DNA calibrators – KS1 B19 and KS2 B19 (in two repeats) and DNA-buffer).

¹ Indicated values include one extra reaction and five controls (2 DNA calibrators – KS1 B19 and KS2 B19 (in two repeats) and DNA-buffer).

² Indicated values include one extra reaction and two controls (Positive Control DNA *Parvovirus* B19 / STI and DNA-buffer).

2. Take the required number of tubes or stripes for amplification of DNA from clinical and control samples.
3. Transfer **15 µl** of prepared reaction mixture into each tube.
4. **Add 10 µl of DNA samples**, obtained at the DNA extraction stage.
5. Carry out the **control amplification reactions**:

For quantitative detection of *Parvovirus* B19 DNA:

- NCA** – Add **10 µl of DNA-buffer** to the tube for Negative Control of Amplification (NCA).
- KS1, KS2** – Add **10 µl of DNA calibrator KS1** into two tubes labeled **KS1** and **10 µl of DNA calibrator KS2** into two tubes labeled **KS2**.
- C–** – Add **10 µl of the sample extracted from the Negative Control (C–) reagent** to the tube labeled C– (Negative control of Extraction).

For qualitative detection of *Parvovirus* B19 DNA:

- NCA** – Add **10 µl of DNA-buffer** to the tube for Negative Control of Amplification (NCA).
- C+** – Add **10 µl of Positive Control DNA *Parvovirus* B19 / STI** to the tube labeled **C+** (Positive Control of Amplification).
- C–** – Add **10 µl of the sample extracted from the Negative Control (C–) reagent** to the tube labeled C– (Negative control of Extraction).

8.2.2. Amplification

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

Table 2

Amlisens-1 amplification program for rotor-type³ and plate-type⁴ instruments

Step	Rotor-type instruments			Plate-type instruments		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
1	95	15 min	1	95	15 min	1
2	95	5 s	5	95	5 s	5
	60	20 s		60	20 s	
	72	15 s		72	15 s	
3	95	5 s	40	95	5 s	40
	60	20 s Fluorescence acquiring		60	30 s Fluorescence acquiring	
	72	15 s		72	15 s	

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores (other channels are enabled when other tests are carried out simultaneously).

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines [2].

³ For example, Rotor-Gene 3000/Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene (QIAGEN, Germany).

⁴ For example for iCycler iQ, iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA).

3. Insert the tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze the 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 two channels:

- The signal of the IC DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the *Parvovirus* B19 DNA amplification product is detected in the channel for the JOE 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 for qualitative detection:

- ***Parvovirus* B19** DNA is **detected** if the *Ct* value determined in the results 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 cross the threshold line in the area of the typical exponential growth of fluorescence.
- ***Parvovirus* B19** DNA is **not detected** in the sample if the *Ct* value is not determined (absent) in the channel for the JOE fluorophore, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary *Ct* value specified in the *Important Product Information Bulletin*.
- The result is **invalid** if the *Ct* value is not determined (absent) in the channel for the JOE fluorophore, whereas the *Ct* value in the channel for the FAM fluorophore is not determined (absent) or greater than the specified boundary *Ct* value. In such cases, the PCR analysis should be repeated starting from the DNA extraction stage. If the same result is obtained in the second run, re-sampling of material is recommended.
- The result is **equivocal** if the *Ct* value determined in the channel for the JOE fluorophore is greater than the boundary *Ct* value specified in the *Important Product Information Bulletin*. In such cases, the sample should be examined in two repeats. If a positive *Ct* value is reproduced in two repeats, then the result is considered positive. If unrepeatable values are obtained then the result is considered equivocal.



Boundary Ct values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

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
C-	DNA extraction	<boundary value	Absent
NCA	PCR	Absent	Absent
C+	PCR	<boundary value	<boundary value

Principle of interpretation for quantitative detection:

Based on obtained boundary Ct values (the fluorescence curve crosses the threshold line set at the specific level) and known calibrator (KS1 B19 and KS2 B19) values indicated in the *Important Product Information Bulletin*, the calibration line will be automatically plotted and the calculation of DNA copies of Positive Control (JOE) and Internal Control (FAM) in the test sample will be automatically performed. Obtained values are used for calculation of concentration of *Parvovirus* B19 DNA in clinical and control samples in accordance with the following formula:

$$\frac{\text{Quantity of Parvovirus B19 DNA (IU/reaction)}}{\text{Quantity of IC STI-87 DNA (copies/reaction)}} \times \text{coefficient A} \times \text{coefficient B (copies/ml)} = \text{IU/ml}$$

$$\text{Coefficient A} = \frac{100}{\text{extraction volume, } \mu\text{l}}$$



Coefficient B (quantity of IC copies/ml) is indicated in the *Important Product Information Bulletin* enclosed in the PCR kit. It is specific for each lot of the PCR kit and can't be used for calculation with PCR kits of other lots

Linear range of the PCR kit is 720 – 9,000,000. If the result is greater than 9,000,000 IU/ml of *Parvovirus* B19 DNA then **result greater than 9,000,000 IU/ml of *Parvovirus* B19 DNA** will be displayed. If the result is less than 720 IU/ml of *Parvovirus* B19 DNA then **result less than 720 IU/ml of *Parvovirus* B19 DNA** will be displayed.

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 4).

Results for controls

Control	Stage for control	Result of amplification	
		FAM	JOE
C–	DNA extraction	$Ct < \text{boundary value}$	Absent
NCA	PCR	Absent	Absent
KS1 B19 KS2 B19	PCR	Ct and calculated concentration are obtained	Ct and calculated concentration are obtained



Boundary Ct values and calibrator concentration values are specified in the *Important Product Information Bulletin* enclosed in the PCR kit.

10. TROUBLESHOOTING

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

1. If an **invalid** result is obtained for a sample, the PCR analysis should be repeated for that sample starting from the DNA extraction stage.
2. Absence of the positive signal for DNA calibrators or Positive Control DNA *Parvovirus* B19 / STI (C_{+B19/STI}) indicates failure of the amplification program or other errors made during preparation or execution of the PCR. Repeat the PCR for all samples once again.
3. If the Ct value is determined for the Negative Control of Extraction (C–) in the channel for the JOE fluorophore or for the Negative Control of Amplification (NCA) in the channels for the FAM and/or JOE fluorophores it indicates contamination of reagents or samples. The result is invalid for all samples. Repeat the analysis and detect and eliminate source of contamination.
4. If a positive result is determined for the sample but its fluorescence curve doesn't have an area of the typical exponential growth of fluorescence (plot is a nearly straight line) it indicates an incorrect level of the threshold line or incorrect calculation of the base line parameters. This result should not be considered positive. If this result is obtained despite the level of the threshold line is accurate repeat the PCR for that sample.

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

11. TRANSPORTATION

AmpliSens® Parvovirus B19-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® Parvovirus B19-FRT** PCR kit (except for PCR-mix-1-

FRT *Parvovirus* B19, PCR-mix-2-FRT, and polymerase (TaqF)) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® Parvovirus B19-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 *Parvovirus* B19, PCR-mix-2-FRT and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C when not in use.



PCR-mix-1-FRT *Parvovirus* B19 is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Clinical material	Nucleic acid extraction kit	PCR kit	Sensitivity, IU/ml ⁵	Linear range, IU/ml
<ul style="list-style-type: none"> - peripheral and umbilical blood, - plasma or serum of peripheral and umbilical blood, - oropharyngeal washes and swabs, - saliva, - cerebrospinal fluid, - bone marrow biopsy samples, - amniotic fluid, - chorionic villi, - placental biopsy samples, - transudate (ascitic fluid) in case of non-immune fetal hydrops) 	RIBO-prep	PCR kit variant FRT-50 F	360	720–9,000,000

13.2. Specificity

The analytical specificity of **AmpliSens® Parvovirus B19-FRT** PCR kit is ensured by 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 PCR kit components do not show any activity on the DNA of the following microorganisms: *Cytomegalovirus hominis*; *Escherichia coli*; *Human adenovirus* B, C, E; *Listeria monocitogenes*; *Mycobacterium tuberculosis*; *Proteus vulgaris*; *Rubella virus*; *Salmonella typhimurium*; *Shigella flexneri*; *Staphylococcus aureus*; *Streptococcus agalactiae*; *Streptococcus pyogenes*; *Toxoplasma gondii*; *Varicella–Zoster virus*; *hepatitis A, B, C, D viruses*; *human herpes virus* types 6, 7; *human immunodeficiency virus*, *herpes simplex virus* types I, II; *Epstein-Barr virus*, *measles virus*, *mumps virus*; *human papillomavirus* types 6, 11, 16, 18, 33, 35 and human DNA.

⁵Number of International Units (IU) of *Parvovirus* B19 DNA per 1 ml of a test sample.

The clinical specificity of the **AmpliSens® Parvovirus B19-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® Parvovirus B19-FRT** PCR kit for qualitative and quantitative detection of *Parvovirus* B19 DNA 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 compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® Parvovirus B19-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

	Catalogue number		Sufficient for
	Batch code		Expiration Date
	<i>In vitro</i> diagnostic medical device		Consult instructions for use
	Version		Keep away from sunlight
	Temperature limitation	NCA	Negative control of amplification
	Manufacturer	C-	Negative control of extraction
	Date of manufacture	C+	Positive control of Amplification
	Authorised representative in the European Community	IC	Internal control
	Caution	KS1 B19, KS2 B19	DNA calibrators

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
30.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
20.09.13 FN	Text	The following clinical material was added: plasma or serum of peripheral or umbilical blood, cerebrospinal fluid, bone marrow biopsy sample, chorionic villi, placental biopsy sample, transudate (ascitic fluid) in case of non-immune fetal hydrops)
		Text was updated in accordance with the valid template
		DNA-sorb-AM and DNA-sorb-B extraction kits are deleted from the reagent kits recommended for the DNA extraction
		Rotor-Gene Q (QIAGEN, Germany) was added
	Misprints and mistakes were corrected	
	9. Data analysis	Formula for calculation of DNA concentration was changed
10. Troubleshooting	Information was corrected and added	
13. Specifications	Analytical sensitivity was changed from 400 copies/ml to 360 IU/ml of <i>Parvovirus</i> B19 DNA	
	Detection linear range was changed from 800 – 10 000 000 copies/ml to 720 – 9 000 000 IU/ml of <i>Parvovirus</i> B19 DNA	
25.02.16 ME	Text	Corrections according to the template. Information about the control of extraction was added
27.06.17 ME	6. Sampling and handling	In the procedure of oropharyngeal swabs sampling the probe with cotton swab was changed to rayon swabs with plastic shafts for oropharyngeal swabs