ECOLI

eSens

CE

IVD

For Professional Use Only

eSens Parvovirus B19 QT PCR kit

REF ES3801B

Instructions for Use

1 INTENDED USE

eSens Parvovirus B19 QT 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.

eSens Parvovirus B19 QT 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).

NOTE: The results of 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.

eSens Parvovirus B19 QT 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.

eSens Parvovirus B19 QT PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by 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.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and dUTP. The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because dUTP is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So, the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	**FAM**	**JOE**
DNA-target	Internal Control STI-87L (IC) DNA	*Primate erythroparvovirus* 1 DNA
Target gene	Artificially synthesized sequence	DNA fragment of structural protein VP1

3 CONTENT

eSens Parvovirus B19 QT PCR kit (ES3801B) includes:

Reagent		Description	Volume, ml	Quantity
PCR-mix-1-FRT *Parvovirus* B19		clear liquid from colorless to light lilac colour	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 *Parvovirus* 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 STI-87 (IC)** during the DNA extraction procedure directly to the sample/lysis mixture

eSens Parvovirus B19 QT PCR kit 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 Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- Disposable polypropylene 0.2 or 0.1-ml tubes:
    - thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
    - 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

eSens Parvovirus B19 QT 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.

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 the temperature not more than minus 16 °C for 6 month at most, or at the temperature not more than minus 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.

   eSens Parvovirus B19 QT PCR kit can be used for the analysis of individual and pooled samples. A minipool should consists of 10 individual samples at most (100 µl of blood plasma obtained from each of 10 samples).

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 µl 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 the temperature not more than minus 16 °C for 1 month at most, or at the temperature not more than minus 68 °C for long-term storage. Only one freeze–thaw cycle of clinical material is allowed.

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 the temperature not more than minus 16 °C for 1 month at most, or at the temperature not more than minus 68 °C for long-term storage.
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 µl 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 the temperature not more than minus 16 °C for 1 month at most, or at the temperature not more than minus 68 °C for long-term storage.

7 WORKING CONDITIONS

eSens Parvovirus B19 QT PCR kit should be used at 18–25 °C.

8 PROTOCOL

8.1 DNA extraction

Any commercial nucleic acid extraction kit, if IVD-CE validated for the indicated specimen types, could be used.

Ecoli Dx, s.r.o. recommends:

- For the manual extraction
  - **RIBO-prep** (K2-9-Et-100-CE)

- For the automatic extraction

  - **ePure Viral Nucleic acid Extraction Kit** (E2003)

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

NOTE: Extract DNA according to the manufacturer's protocol.

8.2 Preparing PCR

8.2.1 Preparing tubes for PCR

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.

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

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 2.

Table 2

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 *Parvovirus* B19**	**PCR-mix-2-FRT**	**Polymerase (TaqF)**
Quantitative detection*	**Qualitative detection****			
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

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

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

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

For <u>quantitative detection</u> it is necessary to carry out 5 controls (2 DNA calibrators – KS1 B19 and KS2 B19 (in two repeats) 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:**

<u>For quantitative detection of *Parvovirus* B19 DNA:</u>

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 3

eSens-1 amplification program

	Rotor-type Instruments (E.g Rotor-Gene Q or equivalent.)			**Plate-type Instruments** (E.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent.)		
Step	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.
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. 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 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 *Technical Sheet*. 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 *Technical Sheet*.
- 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 *Technical Sheet*. 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.

NOTE Boundary Ct values are specified in the *Technical Sheet* enclosed to the PCR kit.

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 4.

Table 4

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 *Technical Sheet*, 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:

$$\frac{\boldsymbol{Quantity\ of\ Parvovirus\ B19\ DNA\ (copies/specimen)}}{\boldsymbol{Quantity\ of\ IC\ STI-87\ DNA\ (copies/specimen)}} \times coefficient\ \mathrm{B} = copies\ DNA\ B19/ml$$

Converting factor to change from *Parvovirus* B19 DNA copies/ml to IU/ml is 0.9 for eSens Parvovirus B19 QT PCR kit.

NOTE: Coefficient B (quantity of IC copies/ml) is indicated in the Technical Sheet 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 5).

Table 5

Results for controls

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

NOTE: Boundary Ct values and calibrator concentration values are specified in the Technical Sheet 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.

11 TRANSPORTATION

eSens Parvovirus B19 QT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12 STABILITY AND STORAGE

All components of the **eSens Parvovirus B19 QT 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 **eSens Parvovirus B19 QT 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.

NOTE: 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**	**Sensitivity IU/ml***	**Linear range, IU/ml**
– 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 ePure Viral Nucleic acid Extraction Kit	360	720–9,000,000

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

13.2 Specificity

The analytical specificity of **eSens Parvovirus B19 QT 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.

The clinical specificity of the **eSens Parvovirus B19 QT PCR kit** was confirmed in laboratory clinical trials.

14 QUALITY CONTROL

The production process, including batch release, is carried out in accordance with an established quality management system certified according to ISO 13485.

15 KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Contains sufficient for <n> tests
LOT	Batch code		Use-by Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limit	NCA	Negative control of amplification
	Manufacturer	C-	Negative control of extraction
	Date of manufacture	C+	Positive control of amplification
EC REP	Authorized representative in the European Community	IC	Internal control
		KS1 B19 KS2 B19	DNA calibrators

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

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