RUO

For Professional Use Only

AmpliSens® *HCV*-genotype-FRT

PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE

AmpliSens® ***HCV*****-genotype-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of hepatitis C virus (*HCV*) genotypes 1a, 1b, 2, 3, and 4 in the clinical materials (peripheral blood plasma) by means of real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

HCV genotypes 1a, 1b, 2, 3, and 4 detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special 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. The real-time PCR monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® *HCV*-genotype-FRT** PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by application of chemically modified polymerase (TaqF) that is activated by heating at 95ºC for 15 min.

HCV genotypes 1a, 1b, 2, 3, and 4 detection includes:

(a) Total RNA extraction from blood plasma simultaneously with the Internal Control sample.

(b) Reverse transcription of cDNA on RNA matrix.

(c) Real-time PCR of *HCV* genotypes 1a, 1b, 2, 3, and 4 cDNA.

3. CONTENT

AmpliSens® *HCV*-genotype-FRT PCR kit is produced in 1 form:

AmpliSens® *HCV*-genotype-FRT PCR kit variant FRT (for use with RG, iQ, Sc)

REF TR-V1-G-2x(RG,iQ,SC)-CE.

AmpliSens® *HCV*-genotype-FRT PCR kit, variant FRT includes:

RIBO-sorb-12 nucleic acid extraction kit:

Reagent	*Description*	*Volume (ml)*	*Amount*
Lysis Solution	colorless, clear liquid	5.8	4 vials
Washing Solution 1	colorless, clear liquid	8.0	4 vials
Washing Solution 3	colorless, clear liquid	15	4 vials
Washing Solution 4	colorless, clear liquid	8.0	4 vials
Sorbent	white suspension	0.4	4 tubes
RNA-buffer	colorless, clear liquid	0.6	4 tubes

RIBO-sorb-12 nucleic acid extraction kit variant 50 is intended for 48 reactions, including controls.

REVERTA-L RT reagent kit variant 50 includes:

Reagent	*Description*	*Volume (ml)*	*Quantity*
RT-G-mix-1	colorless, clear liquid	0.01	5 tubes
RT-mix	colorless, clear liquid	0.125	5 tubes
Revertase (MMlv)	colorless, clear liquid	0.03	1 tube
DNA-buffer	colorless, clear liquid	1.2	1 tube

REVERTA-L RT reagent kit is intended for 60 reverse transcription reactions, including controls.

AmpliSens® *HCV*-genotype-FRT PCR kit variant FRT includes:

Reagent	*Description*	*Volume (ml)*	*Amount*
PCR-mix-1-FRT *HCV* genotypes 1b/3	colorless, clear liquid	0.11	4 tubes
PCR-mix-1-FRT *HCV* genotypes 1a/2	colorless, clear liquid	0.11	4 tubes
PCR-mix-1-FRT *HCV* genotype 4/IC	colorless, clear liquid	0.11	4 tubes
RT-PCR-mix-2-FEP/FRT	colorless, clear liquid	0.3	4 tubes
Polymerase (TaqF)	colorless, clear liquid	0.02	4 tubes
Positive Control cDNA *HCV* genotypes 1b/3 ($C+_{1b/3}$)	colorless, clear liquid	0.1	1 tube
Positive Control cDNA *HCV* genotypes 1a/2 ($C+_{1a/2}$)	colorless, clear liquid	0.1	1 tube
Positive Control cDNA *HCV* genotype 4 ($C+_4$)	colorless, clear liquid	0.1	1 tube
TE-buffer	colorless, clear liquid	0.5	1 tube
Negative Control (C-)*	colorless, clear liquid	0.5	1 tube
Internal Control STI-248-rec (IC)**	colorless, clear liquid	0.13	4 tubes

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

must be used in the extraction as Internal Control (see “RIBO-sorb-12” or “RIBO-prep” **REF K2-9-Et-50-CE protocols)

AmpliSens® *HCV*-genotype-FRT PCR kit is intended for 39 tests (156 amplification reactions), including controls.

4. ADDITIONAL REQUIREMENTS

For RNA extraction:

- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers up to 200 µl.
- Tube racks.
- Vortex mixer.
- Desktop centrifuge up to 16,000 g (suitable for Eppendorf tubes).
- PCR box.
- Thermostat with working temperature +25 °C to +100 °C.
- Vacuum aspirator with flask for removing a supernatant.
- Disposable polypropylene 1.5 ml tubes (for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer with temperature below minus 16 °C.
- Waste bin for used tips.

For reverse transcription, PCR-amplification:

- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters up to 200 µl.
- Tube racks.
- Vortex mixer.
- Thermostat with working temperature 25 – 100 °C.
- PCR box.
- Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); iQ5 or iQ iCycler (BioRad, USA); SmartCycler II (Cepheid, USA) or equivalent instrument.
- Disposable polypropylene microtubes for PCR with 0.5 (0.2) ml capacity (for example, Axygen, Cephied USA) suitable for real-time PCR instrument used.
- Refrigerator for 2 – 8 °C.
- Deep-freezer with temperature below minus 16 °C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes and mucose membranes. If any of 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 the techniques of DNA amplification.
- Workflow in the laboratory must proceed in a unidirectional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.

 Xn	**Lysis Solution** **Washing Solution 1**	Contains guanidine thiocyonate. Guanidine thiocyonate is harmful if inhaled or comes in contact with skin or if swallowed. Contact with acid releases toxic gas. Harmful (Xn). Risk and safety phrases:* R20/21/22-32, S13-26-36-46
	**Washing Solution 3** **Washing Solution 4**	Contains ethanol: flammable. Risk phrase:* R10

*R10: Flammable;
R20/21/22: Harmful by inhalation, in contact with skin and if swallowed;
R32: Contact with acids liberates very toxic gas;
S13: Keep away from food, drink and animal feeding stuffs;
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice;
S36: Wear suitable protective clothing;

S36/37: Wear suitable protective clothing and gloves;
S46: If swallowed, seek medical advice immediately and show the container or label.

Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

Obtaining of biological material samples for PCR-analysis, transportation, and storage are described in detail in manufacturer's handbook [1]. It is recommended that this handbook is read before starting the work.

AmpliSens® *HCV*-genotype-FRT PCR kit is intended for the analysis of RNA extracted with RNA extraction kits from:

— *Peripheral blood plasma*

Take a blood sample in a tube with 3% EDTA solution (1 : 20) after overnight fasting. Invert closed tube several times to ensure adequate mixing. Remove and transfer plasma specimen in a new tube within 6 h from the time of blood taking. To do this, centrifuge the tube with blood at 800 – 1600 rpm for 20 min.

Storage of plasma samples:

– from 2 °C to 8 °C for up to 3 days;

– at or below minus 16 °C for a long time.

7. WORKING CONDITIONS

AmpliSens® *HPV* HCR genotype-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA extraction

It's recommended that the following nucleic acid extraction kits are used:

- "RIBO-prep", **REF** K2-9-Et-50-CE (follow the instructions of the manufacturer);
- "RIBO-sorb-12" (extraction is described below).

RNase-free and DNase-free plastic ware should be used only.

"RIBO-sorb-12" extraction instructions

The volume of a sample required for RNA extraction is **0.1 ml**.

1. Warm up **Lysis Solution** and **Washing Solution 1** (if stored at 2 – 8 ºC) at 56 ºC until the ice crystals disappear.
2. Prepare the required number of 1.5 ml tubes including the tube for Negative Control of Extraction (**C-**).
3. Add **450 µl of Lysis Solution** and **10 µl of IC STI-248-rec** per each tube. Label the

tubes.

4. Add **100 µl of plasma sample** per each tube containing Lysis Solution and IC. Close the tubes and vortex. Make sure there are no drops on the walls of the tubes; otherwise, centrifuge briefly.
5. Add 100 **µl of Negative Control** to the tube intended for the Negative Control of Extraction (C-). Close the tubes and vortex. Make sure there are no drops on the walls of the tubes; otherwise, centrifuge briefly.
6. Thoroughly resuspend **Sorbent** with the vortex. Add **25 µl** of resuspended sorbent to each test tube. Use a new tip for every tube.
7. Vortex the tubes and incubate them at room temperature for 10 min with stirring every 2 min.
8. Centrifuge the tubes at 10,000 g for 1 min.
9. Remove and discard the supernatant from the tubes with vacuum aspirator. Use a new tip for every tube.
10. Add **500 µl** of **Washing Solution 1** to each tube. Vortex thoroughly (until the sorbent is fully resuspended) then centrifuge at 10,000 g for 1 min. Remove and discard the supernatant with vacuum aspirator. Use a new tip for every tube.
11. Add **500 µl** of **Washing Solution 3** to each tube. Vortex thoroughly (until sorbent is fully resuspended) then centrifuge at 10,000 g for 1 min. Remove and discard the supernatant with vacuum aspirator. Use a new tip for every tube.
12. Repeat step 11.
13. Add **500 µl** of **Washing Solution 4** to each tube. Vortex thoroughly (until sorbent is fully resuspended) then centrifuge at 10,000 g for 1 min. Remove and discard the supernatant with vacuum aspirator. Use a new tip for every tube.
14. Incubate the tubes at 56 °C for 15 min to dry the sorbent. Make sure the tubes are open while incubating.
15. Add **50 µl of RNA-buffer** per each tube. Resuspend the sorbent in RNA-buffer, incubate at 56 °C for 5 min, and then vortex. To sediment the sorbent, centrifuge the tubes at 10,000 g for 2 min.

Once RNA is extracted, it must be processed within 20 – 30 minutes.
Do not store RNA samples.

8.2 Reverse transcription

It's recommended that the following reverse transcription reagent kits are used:

- "REVERTA-L", **REF** K3-4-50-CE (the procedure is describe below).

RNase-free and DNase-free plastic ware should be used only.

The total reaction volume is **20 µl**, the volume of RNA sample is **10 µl**.

1. Take the required number of 0.2 ml tubes.
2. Prepare reaction mixture for 12 reactions:
   2.1. Add **5 µl** of **RT-G-mix-1** to the tube with **RT-mix** and thoroughly mix by vortexing. Make sure there are no drops on the walls of the tubes; otherwise, centrifuge briefly.
   2.2. Add **6 µl** of **Revertase (MMlv)** to the tube with the reaction mixture, pipette 5 times, and vortex. Make sure there are no drops on the walls of the tubes; otherwise, centrifuge briefly.
3. Transfer **10 µl** of the prepared mixture per each tube.
4. Add **10 µl of RNA-sample** per each tube with the reaction mixture. Carefully mix.
5. Place the tubes in a thermostat (or a thermal cycler*) and incubate at 37 °C for 30 min.
6. Dilute the cDNA sample obtained during reverse transcription for further PCR test. To do this, add **20 µl of DNA-buffer** to the tube with **20 µl of cDNA sample** and carefully mix by pipetting (10 times).

Storage of cDNA samples:

– at or below minus 16 °C for 1 week;
– at or below minus 68 °C for 1 year.

*If Rotor-Gene 3000/6000 is used for reverse transcription, program the instrument as follows:

1. Click on the **New** button in the program main menu.
2. Select the **Advanced** template in the opened window and indicate the **Dual Labeled Probe/Hydrolysis Probe** option. Click on the **New** button.
3. Select the **36-Well Rotor** and **No domed tubes** in the opened window. Press **Next**.
4. Set the reaction volume as **20 µl** and select the operator. Press **Next**.
5. In the opened window specify the experiment temperature profile. To do this, click on the **Edit profile** button:
   – select the **Hold** parameter. Enter **37 °C** and **30 min**.
   – select the **Cycling** parameter and delete it by clicking the **Remove** button.
6. Click **OK**.
7. In the **New Run Wizard** window press the **Calibrate/Gain Optimization…** button. Make sure that calibration is not activated (no check mark) in the opened window; otherwise, cancel calibration. If fluorescence channels are enabled, press the **Remove All** button. Click **Close**.
8. Press the **Next** and then **Start run** button to execute the program.
9. Name the experiment and save it to the disc (all data will be saved in this file).

8.3 Preparing the PCR

The total reaction volume is **25 µl**, the volume of cDNA sample is **12.5 µl**.

8.3.1 Preparing tubes for PCR

1. Take the required number of PCR tubes.
2. Prepare the following reaction mixtures: **"1b/3", "1a/2", and "4/IC"**. To do this, add **65 µl of RT-PCR-mix-2-FEP/FRT** and **6 µl of polymerase (TaqF)** per each tube with PCR-mix-1-FRT *HCV* genotypes 1b/3, PCR-mix-1-FRT *HCV* genotypes 1a/2, PCR-mix-1-FRT *HCV* genotype 4/IC. Thoroughly vortex. Make sure there are no drops on the walls of the tubes; otherwise, centrifuge briefly.
3. Transfer **12.5 µl or prepared mixture** to the PCR tubes. Discard the unused mixture.
4. Add **12.5 µl of cDNA samples** obtained from clinical or control samples at the stage of RNA extraction and reverse transcription to the tubes.
5. Carry out control amplification reactions:

NCA – add **12.5 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification).

$C+_{1b/3}$ – Add **12.5 µl** of **Positive Control cDNA *HCV* genotypes 1b/3** to the tube with **"1b/3"** reaction mixture labeled **$C+_{1b/3}$** (Positive Control of Amplification).

$C+_{1a/2}$ – Add **12.5 µl** of **Positive Control cDNA *HCV* genotypes 1a/2** to the tube with **"1a/2"** reaction mixture labeled **$C+_{1a/2}$** (Positive Control of Amplification).

$C+_{4}$ – Add **12.5 µl** of **Positive Control cDNA *HCV* genotype 4** to the tube with **"4/IC"** reaction mixture labeled **$C+_{4}$** (Positive Control of Amplification).

8.3.2. Amplification

Create a temperature profile on your Real-time instrument as follows:

Amplification program for Rotor-Gene 3000/6000

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*
Hold	95	15 min	–	1
Cycling	95	20 sec	–	45
	60	40 sec	FAM/Green, JOE/Yellow	

Adjust the fluorescence channel sensitivity according to Appendix 1.

Amplification program for iQ5 and iQ iCycler

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*
1	95	15 min	–	1
2	95	20 sec	–	45
	60	1 min	FAM, HEX	

Adjust the fluorescence channel sensitivity according to Appendix 2.

Amplification program for SmartCyclerII

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*
Hold	95	15 min	–	1
2-Temperature Cycle	95	20 sec	–	45
	60	40 sec	FAM, Cy3	

Adjust the fluorescence channel sensitivity according to Appendix 3.

9. DATA ANALYSIS

Amplification products of the Internal Control and the *HCV* RNA fragments are analyzed within the test. Matching of the fluorescence channels with the *HCV* genotypes is specified in the table below.

Channel	Reaction mixture		
	1b/3	**1a/2**	**4/IC**
FAM/Green	1b	1a	IC
JOE/Yellow/HEX/Cy3	3	2	4

The *HCV* genotype found in a sample should be confirmed by the results of amplification obtained from three test tubes (with PCR-mix-1-FRT *HCV* genotypes 1b/3, PCR-mix-1-FRT *HCV* genotypes 1a/2, and PCR-mix-1-FRT *HCV* genotype 4/IC).

***HCV* genotype is not detected in a sample** if only IC signal is registered.

Results interpretation

The results are interpreted by the software of the used instrument by the crossing (or no crossing) of the fluorescence curve with the threshold line that corresponds to the presence (or absence) of Ct value (or “Pos” result, for SmartCycler II only) in the result grid.

Results are accepted as relevant if both positive and negative controls of amplification along with the negative control of extraction are passed (see tables below).

Results of controls

Control	Stage for controls	Reaction mixture						Interpretation
		1b/3		1a/2		4/IC		
		FAM/ Green	JOE/ Yellow	FAM/ Green	JOE/ Yellow	FAM/ Green	JOE/ Yellow	
C-	RNA extraction	–	–	–	–	<Ct*	–	OK
NCA	PCR	–	–	–	–	–	–	OK
$C+_{1b/3}$	PCR	<Ct*	<Ct*					OK
$C+_{1a/2}$	PCR			<Ct*	<Ct*			OK
$C+_{4}$	PCR					–	<Ct*	OK

*For Ct values see **Appendix** enclosed to instruction manual.

1. If only the IC signal appears in the sample, the **“not typed”** result is displayed.
2. If the signal corresponding to a certain genotype appears in the sample, the **“genotype…”** result is displayed.

3. If the signals corresponding to two or more genotypes appear in a sample, multiple genotypes are displayed. However, there are two exceptions:
   – The Ct value corresponding to genotype 4 is less than that of genotype 1 by 15 cycles and more. In this case, the results corresponding to 1a and 1b genotypes are not taken into account and the **“genotype 4”** result is displayed. If the signal corresponding to genotype 2 or 3 appears in the same sample, then **“genotypes 2, 4”** or **“genotypes 3, 4”**, respectively, are displayed.
   – signals corresponding to genotypes 1a and 1b appear simultaneously in a sample. In this case the **“genotype 1”** result is displayed.
4. If the signals of all genotypes are absent in the sample while the signal of the IC is more than **38** cycles or absent (for Rotor-Gene 3000/6000), more than **40** cycles or absent (for iQ iCycler or iQ5), or absent (for SmartCyclerII), then the test should be repeated starting from the RNA extraction.

10. TROUBLESHOOTING

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

1. if the Ct value of the Negative Control of Extraction (C-) detected with **“4/IC”** mixture in the FAM/Green channel (detection of the Internal Control) is:
   – more than **38** or absent (for Rotor-Gene 3000/6000);
   – more than **40** or absent (for iQ iCycler and iQ5);
   – absent (for SmartCyclerII).
2. if th Ct value of at least one Positive Control of Amplification ($C+_{1b/3}$, $C+_{1a/2}$, or $C+_{4}$) is more than the value specified in the “Results of controls” table or absent (see the above tables). It can indicate errors in PCR conducting. The PCR should be repeated.
3. if any Ct value is detected for Negative Control of Extraction, C-, (except for the Ct obtained in the FAM/Green channel with **“4/IC”** mixture) or for Positive Control of Amplification, $C+_{4}$, (only in the FAM/Green channel). It indicates the contamination of reagents or samples. In this case the results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis, and also to take measures to detect and eliminate the source of contamination.
4. if any Ct value is detected for Negative Control of Amplification, NCA, in any of the channels with any PCR-mix-1-FRT. It indicates the contamination of reagents or samples. In this case the results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis and take measures to detect and eliminate the source of contamination.

11. TRANSPORTATION

AmpliSens® *HCV*-genotype-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® *HCV*-genotype-FRT** PCR kit should be stored as specified below when not in use. All components 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.

Store at 2 – 8 °C

RIBO-sorb-12
PCR kit (**except for**
PCR-mix-1-FRT *HCV* genotypes 1b/3,
PCR-mix-1-FRT *HCV* genotypes 1a/2,
PCR-mix-1-FRT *HCV* genotype 4/IC,
RT-PCR-mix-2-FEP/FRT,
polymerase (TaqF))

Store at temperature
from minus 24 to minus 16 °C
REVERTA-L

PCR-mix-1-FRT *HCV* genotypes 1b/3,
PCR-mix-1-FRT *HCV* genotypes 1a/2,
PCR-mix-1-FRT *HCV* genotype 4/IC,
RT-PCR-mix-2-FEP/FRT,
polymerase (TaqF) (from PCR kit)

PCR-mix-1-FRT *HCV* genotypes 1b/3, PCR-mix-1-FRT *HCV* genotypes 1a/2, and PCR-mix-1-FRT *HCV* genotype 4/IC are to be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *HCV*-genotype-FRT** PCR kit is no less than 1×10^3 International Units per 1 ml of sample (IU/ml).

The claimed analytical features of **AmpliSens® *HCV*-genotype-FRT** PCR kit are guaranteed only when an additional reagent kit, “RIBO-sorb-12” or “RIBO-prep” (manufactured by FBIS CRIE), is used.

13.2. Specificity

Specificity of **AmpliSens® *HCV*-genotype-FRT** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. Specificity of **AmpliSens ® *HCV*-genotype-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, transportation, storage of clinical material for PCR diagnostics”, developed by Federal Budget Institution of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

15. QUALITY CONTROL

In compliance with Federal Budget Institution of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens®** ***HCV*-genotype-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
FBIS CRIE	Federal Budget Institution of Science “Central Research Institute for Epidemiology”	**C+**	Positive control of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
18.07.10	Text	Reference number was changed from TR-V1-G-2x(RG,iQ) to TR-V1-G-2x(RG,iQ)-CE
	Page footer	
28.02.11 LA	Cover page	The phrase “For Professional Use Only” was added
	Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” was added
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of reagents before and after the first use was added
		Information that PCR-mixes-1-FRT are to be kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
05.07.11 LA	Cover page, text	The name of Institution was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
10.10.11 LA	Text	Reference number was changed from TR-V1-G-2x(RG,iQ)-CE to TR-V1-G-2x(RG,iQ,SC)-CE
	Page footer	