AmpliSens® *HCV*-Monitor-L PCR kit

CE 1023

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

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

1. INTENDED USE

AmpliSens® *HCV*-Monitor-L PCR kit is an *in vitro* nucleic acid amplification test for quantitative detection of *hepatitis C virus* (*HCV*) RNA in biological material using real-time hybridization-fluorescence detection of amplified products. The material for PCR is RNA-samples extracted from blood plasma.

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

2. PRINCIPLE OF PCR DETECTION

Principle of testing is based on the RNA extraction from the samples of test material with the exogenous internal control (IC) sample (Internal Control 1L), RNA reverse transcription and simultaneous amplification of cDNA fragments with hybridization-fluorescence detection. Exogenous internal control (Internal Control 1L) allows to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition.

HCV detection by the polymerase chain reaction (PCR) is based on the extraction of RNA from blood plasma, reverse transcription reaction of the RNA and the amplification of cDNA corresponding to a specific region using specific *HCV* 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 of the reaction tubes after the PCR run.

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

Table 1

Channel for fluorophore	FAM	JOE
cDNA-target	IC cDNA	*HCV* cDNA
Target gene	Artificially synthesized sequence	5'UTR

3. CONTENT

AmpliSens® *HCV*-Monitor-L PCR kit is produced in 1 form:

variant FRT-L REF H-4002-1-14-CE.

Variant FRT-L includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix *HCV*-Lyo	white powder	–	96 tubes of 0.2 ml
Internal Control 1L	white powder	–	4 tubes
Positive Control 1L *HCV*	white powder	–	4 tubes
Positive Control 2L *HCV*	white powder	–	4 tubes
Calibrator C1L *HCV*	white powder	–	4 tubes
Calibrator C2L *HCV*	white powder	–	4 tubes
Negative Control (C–)	clear liquid from colorless to straw-yellow colour (flake sediment is allowed)	5.0	5 tubes
TE-buffer	colorless clear liquid	0.2	4 tubes

Variant FRT-L is intended for 96 reactions, including controls and calibrators.

4. ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- Sterile pipette tips with filters up to 100 µl, 200 µl and up to 1000 µl.
- Tube racks.
- Vortex mixer.
- Desktop microcentrifuge up to 12,000 g (suitable for Eppendorf tubes).
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); CFX96 (Bio-Rad, USA), DTprime ("DNA-Technology", Russia)).
- Disposable polypropylene tubes:
  a) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps with optical transparent 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.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

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 the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- 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 inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section "Content").
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- 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

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

AmpliSens® *HCV*-Monitor-L PCR kit is intended for the analysis of RNA extracted with RNA extraction kits from the biological material (blood plasma).

Sampling

Blood samples are collected in the morning in the fasting state into the tube with EDTA solution as the anticoagulant. Several times invert the closed tubes to ensure proper mixing. To collect plasma, centrifuge the tubes with blood at 800–1600 g for 20 min within 6 h after blood sampling. Remove obtained plasma and transfer to the new tubes.

Blood plasma samples can be stored:

— at the temperature from 2 to 8 °C – for 3 days,
— at the temperature from minus 24 to minus 16 °C – for 1 year,
— at the temperature no more than minus 68 °C – for a long time.

The blood serum may also be used in some cases. The analytical sensitivity of the reagent kit is retained for this material; however, the clinical sensitivity may be significantly decreased as a result of viral particles precipitation during blood clot retraction.

Blood serum samples can be stored:

— at the temperature from 2 to 8 °C – for 3 days,
— at the temperature no more than minus 68 °C – for a long time.

Pretreatment

Pretreatment of *blood plasma* and *blood serum samples* is not required.

Interfering substances and limitations of using test material samples

The next samples are inapplicable for analysis:

— the whole blood samples, collected in the tubes with heparin as anticoagulant.

7. WORKING CONDITIONS

AmpliSens® *HCV*-Monitor-L PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. RNA Extraction

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

- RIBO-prep,
- MAGNO-sorb.

The RNA extraction of each test sample is carried out in the presence of Internal Control (see 8.1.1 *Rehydration of lyophilized control samples*).

Add 10 µl of rehydrated **Internal Control 1L** to the tubes with test samples, control samples and calibrators.

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

C– – Add **Negative Control (C–)** to tube labeled **C–** (Negative Control of Extraction).

PCE1 – Add rehydrated **Positive Control 1L *HCV*** to tube labeled **PCE1** (Positive Control of Extraction).

PCE2 – Add rehydrated **Positive Control 2L *HCV*** to tube labeled **PCE2** (Positive Control of Extraction).

Extract RNA according to the manufacturer's protocol taking into account next additions and improvements:

NOTE:
- The volume of rehydrated control samples and Negative Control (C–) is the same as the extraction volume specified in the Instruction Manual for the extraction reagent kit:
  - **<u>If using RIBO-prep kit</u>** the volume of the test sample is 100 µl.
  - **<u>If using the MAGNO-sorb kit</u>** the volume of the test sample is 200 or 1000 µl.
- The elution volume should be **100 µl** (for recommended nucleic acid extraction kits).

NOTE: Perform calibration according to 8.1.2 *Calibration.*

8.1.1 Rehydration of lyophilized control samples

1. Take the required number of the lyophilized control samples. Sediment the content of the tubes by vortex, carefully open them and avoiding spraying of the content, add **Negative Control (C–)** according to the table.

Control	Volume of Negative Control (C–), µl
Positive Control 1L *HCV*	1200
Positive Control 2L *HCV*	1200
Calibrator C1L *HCV*	1200
Calibrator C2L *HCV*	1200
Internal Control 1L	300

2. Tightly close the tubes and leave them for 2 min at room temperature, stirring occasionally by vortex.
3. After full dissolution sediment the content of the tubes on the vortex for 3-5 s.

NOTE: Rehydrated **Calibrator C1L *HCV***, **Calibrator C2L *HCV***, **Internal Control 1L**, **Positive Control 1L *HCV*** and **Positive Control 2L *HCV*** are to be stored at 2-8 °C for no longer than 3 months.

8.1.2 Calibration

It is necessary to analyze additional points <u>at the first run</u> of PCR kit for the <u>given lot</u>: **Calibrator C1L *HCV*** and **Calibrator C2L *HCV*** in at least three replicates each (4 replicates are recommended), beginning from the extraction stage. The extraction procedure for calibrators is similar to the extraction procedure for the test samples. After successful calibration, it can be used within 6 months.

Analyze the points for calibration:

C1 – Add rehydrated **Calibrator C1L *HCV*** to 3 or 4 tubes labeled **C1**

C2 – Add rehydrated **Calibrator C2L *HCV*** to 3 or 4 tubes labeled **C2**

NOTE: The volume of rehydrated calibrators is the same as the extraction volume specified in the *Instruction Manual* for the extraction reagent kit

8.2 Preparing reverse transcription and PCR

The total reaction volume is **50 µl**, the volume of RNA sample is **50 µl**.

8.2.1 Preparing tubes

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

1. Take the required number of the tubes with ready-to-use lyophilized reaction mixture **PCR-mix *HCV*-Lyo** for amplification from test and control samples (3 controls of extraction in 1 replicate, 2 calibrators in 3 or 4 replicates in case of calibration).
2. Add **50 µl** of **RNA samples**, obtained by extraction from test samples, controls and calibrators (in case of calibration).

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

NOTE: It is also necessary to carry out Negative Control of Amplification (NCA) at suspicion on possible contamination.

NCA – Add **50 µl** of **TE-buffer** to the tube with lyophilized reaction mixture

NOTE: Avoid transferring of the sorbent together with the RNA sample in case of extraction using reagent kit for extraction on silica gel or magnetic separation.

8.2.2. Reverse transcription and amplification

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

Table 2

AmpliSens-2 RG amplification program for rotor-type instruments[1]

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
Hold	50	15 min	–	1
Hold 2	95	15 min	–	1
Cycling	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
Cycling 2	95	5 s	–	40
	60	20 s	FAM, JOE	
	72	15 s	–	

Table 3

AmpliSens-2 iQ amplification program for plate-type instruments[2]

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	50	15 min	–	1
2	95	15 min	–	1
3	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
4	95	5 s	–	40
	60	30 s	FAM, JOE	
	72	15 s	–	

NOTE: Any combination of the tests (for example simultaneously with *HDV* detection tests; *HCV* genotyping and etc.) can be performed in one instrument simultaneously with the use of either AmpliSens-2 RG or AmpliSens-2 iQ programs.

NOTE: If several tests in "multiprime" format are carried out simultaneously, the detection is enabled in other used channels except for the specified ones.

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

NOTE: 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. Analyse 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:

Channel for the fluorophore	FAM	JOE
Signal registration, indicating the amplification product accumulation	IC cDNA	*HCV* cDNA

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 cDNA sample in the corresponding column of the results grid.

Based on the obtained *Ct* values for *HCV* calibrators (C1 and C2) and specified concentration values a calibration line is plotted. The efficiency of amplification should fall in the range of 0.85-1.15, and the correlation coefficient of the calibration line should be more than 0.95. If the calibration line meets the abovementioned requirements, then the equation of this calibration line can be used for calculation of *HCV* RNA concentration and results interpretation for 6 months.

For calculation of *HCV* RNA concentration, the median *Ct* value of Internal control (IC) (*Ct* IC_{med}) is calculated for all samples (except for NCA) at the first step.

NOTE: Calculated value of the median *Ct* value of Internal control (IC) (*Ct* IC_{med}) should be no more than *Ct* value for Internal control specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

If *Ct* value of Internal control differ from the median *Ct* value of Internal control (IC) by more than 3, the *Ct* value of Internal control for this sample should be excluded from analysis and the median value should be recalculated. The calculation of *HCV* RNA concentration for this sample is not carried out, the sample is invalid.

If *Ct* value of Internal control differs from median value by more than 1 and less than 3 the following correction for *Ct HCV* is introduced:

$$Ct\ HCV_{corrected} = Ct\ HCV + (Ct\ IC_{med} - Ct\ IC)$$

The concentration of *HCV* RNA in this sample is recalculated according to the equation for calibration line (y = kx + b) taking into account corrected *Ct* value for *HCV*.

The correction of *Ct* value for *HCV* is not introduced for samples for which *Ct* value of Internal control differ from the median value by less than 1.

Table 4

Results interpretation for the test samples

Result	Interpretation
HCV RNA is not detected	The *Ct* value of Internal control does not differ from median value by more than 2 and the *Ct* value for *HCV* RNA is absent. The result is interpreted ***HCV* RNA is not detected**
less than 200 IU/ml or less than 100 IU/ml or less than 20 IU/ml	*HCV* RNA is detected in concentration less than the lower limit of the measurement range. If the result is less than 200 IU/ml in case of extraction from 100 µl of the sample, less than 100 IU/ml in case of extraction from 200 µl of the sample, less than 20 IU/ml in case of extraction from 1 ml of the sample, it is interpreted as **less than 200, less than 100, less than 20 IU of *HCV* RNA/ml,** respectively
$X.XX*10^X$ IU/ml	Calculated value IU/ml falls in the measurement range. The result is interpreted as ***HCV* RNA was detected in concentration $X.XX*10^X$ IU/ml**
greater than $1.00*10^8$ IU/ml	Calculated value IU/ml is greater than upper limit if measurement range. The result is interpreted as **greater than $1.00*10^8$ IU of *HCV* RNA/ml.** If more precise quantitative result is required, dilute the *HCV* sample with Negative Control (C–) (e.g. 10 times) and repeat the analysis. The result obtained by repeated analysis must be multiplied by the coefficient of the sample dilution
Invalid	The *Ct* value of Internal control differs from median value by more than 2 and the *Ct* value for *HCV* is absent. The PCR analysis (beginning with the RNA extraction stage) should be repeated
	The *Ct* value for *HCV* RNA is determined and the *Ct* value for Internal Control differs from median by more than 3. The PCR analysis (beginning with the RNA extraction stage) should be repeated

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification, Positive and Negative Controls of extraction as well as for calibrators are correct.

[1] For example, Rotor-Gene Q (QIAGEN).

[2] For example, CFX (Bio-Rad, USA).

Table 5

Results for controls

Control	Stage for control	Results in the channel for fluorophore	
		FAM	JOE
C–	RNA extraction, RT-PCR	*Ct* value is determined in the ±3 range of the median *Ct* value of Internal control	*Ct* value is absent
PCE1	RNA extraction, RT-PCR		Concentration value falls in the ± 0,5 Lg range of the value specified in the *Important Product Information Bulletin*
PCE2	RNA extraction, RT-PCR		Concentration value falls in the ± 0,5 Lg range of the value specified in the *Important Product Information Bulletin*
C1	RNA extraction, RT-PCR		The *Ct* value and calculated concentration are determined. The efficiency of PCR falls into the 0.85-1.15 range. The coefficient R^2 is no less than 0.95
C2	RNA extraction, RT-PCR		
NCA	RT-PCR	*Ct* value is absent	*Ct* value is absent

NOTE: Concentration values are calculated taking into account the correction of *Ct* value for *HCV* by *Ct* value of Internal control using the equation for calibration line.

10. TROUBLESHOOTING

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

1. The *Ct* value is absent for calibrators (C1 and C2) in the channels for FAM, JOE fluorophores. Check the correctness of set values of calibrators in accordance with the *Important Product Information Bulletin*. If the improper result has been obtained again the amplification and detection for all the samples including calibrators should be repeated.
2. The correlation coefficient R^2 is less than 0.95 when plotting the calibration curve. Check the correctness of set concentrations of calibrators in accordance with the *Important Product Information Bulletin*. If the improper result has been obtained again the amplification and detection for all the samples including calibrators should be repeated.
3. The efficiency ***E*** is less than 0.85 or greater than 1.15 when plotting the calibration line. Check the correctness of set concentrations of calibrators in accordance with the *Important Product Information Bulletin* and the correctness of selected level of the threshold line. If set concentrations of calibrators and the threshold line level are correct but the efficiency does not fit in the required range, then the amplification and detection for all the samples including calibrators should be repeated.
4. The *Ct* value is absent for the Positive Control of Extraction (PCE1, PCE2) in the channels for the FAM and JOE fluorophores. The PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples in which *HCV* RNA was not detected.
5. The calculated concentrations of PCE1 and PCE2 do not fall in ± 0,5 Lg range of the value specified in the *Important Product Information Bulletin*. The PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples.
6. If the *Ct* value is determined for the Negative Control of Extraction (C–) in the channel for JOE fluorophore. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples in which specific RNA was detected.
7. The *Ct* value determined for the Negative Control of Extraction (C–) in the channel for FAM fluorophore is more than 3 of the median *Ct* value for internal control. The PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples.
8. The *Ct* value is determined for the Negative Control of Amplification (NCA) in the channel for JOE fluorophore. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the amplification stage) should be repeated for all samples in which specific RNA was detected.
9. The *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level or parameters of base line calculation. If the result has been obtained with the correct level of threshold line (base line), the amplification and detection should be repeated for this sample.

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

11. TRANSPORTATION

AmpliSens® *HCV*-Monitor-L PCR kit should be transported at 2–8 °C.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *HCV*-Monitor-L** PCR kit are to be stored at 2-8 °C when not in use. All components of the **AmpliSens® *HCV*-Monitor-L** PCR kit are stable until the expiration date stated on the label. The shelf life of the reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix *HCV*-Lyo, Internal Control 1L, Positive Control 1L *HCV,* Positive Control 2L *HCV,* Calibrator C1L *HCV* and Calibrator C2L *HCV* are to be kept (before solution) in packages with a desiccant.

NOTE: Rehydrated Calibrator C1L *HCV*, Calibrator C2L *HCV*, Internal control 1L, Positive Control 1L *HCV* and Positive Control 2L *HCV* are to be stored at 2-8 °C for no longer than 3 months.

NOTE: PCR-mix *HCV*-Lyo is to be kept away from light.

13. SPECIFICATIONS

13.1. Detection limit and linear measurement range

PCR kit	RNA extraction kit	Volume of sample for extraction, µl	Detection limit, IU/ml	Linear measurement range, IU/ml
Variant FRT-L	Recommended by the manufacturer (see *8.1. RNA extraction*)	100	70	200 – 100 000 000
		200	35	100 – 100 000 000
		1000	7	20 – 100 000 000

The claimed features are achieved while respecting the rules specified in the section "*Sampling and Handling*".

Note – If it is necessary to obtain results expressed in copies/ml, the results measured in International Units (IU/ml) should be multiplied by 4 (i.e. 1 IU = 4 copies, 1 copy = 0.25 IU). However, it should be remembered that unlike the International Units (IU/ml), the values expressed in copies/ml may differ significantly depending on the manufacturer of reagent kits.

13.2. Analytical specificity

The analytical specificity of **AmpliSens® *HCV*-Monitor-L** PCR kit is ensured by the selection of specific primers and probes as well as reaction conditions. The primers and probes were checked for possible homologies to all sequences published in the gene banks by sequence comparison analysis.

The analytical specificity is also ensured by the addition of the genomic DNA/RNA of the following organisms and viruses to the reaction: hepatitis A virus (*HAV*); hepatitis B virus (*HBV*); hepatitis D virus (*HDV*); human immunodeficiency virus (*HIV*); cytomegalovirus (*CMV*); Epstein-Barr virus (*EBV*); herpes simplex virus types 1 and 2 (*HSV* I, II); *varicella-zoster* virus (*VZV*); human herpes virus types 6 and 8; parvovirus B19; tick-borne encephalitis virus (*TBEV*); West Nile encephalitis virus (*WNV*); adenovirus types 2, 3, and 7; *Escherichia coli*; *Staphylococcus aureus; Streptococcus pyogenes; Streptococcus agalactiae;* and *Homo sapiens*.

No cross-reactions were observed for the abovementioned organisms and viruses.

The clinical specificity of **AmpliSens® *HCV*-Monitor-L** PCR kit was confirmed in laboratory clinical trials.

13.3. Diagnostic characteristics

111 positive and 108 negative samples of biological material (blood plasma) were used for diagnostic sensitivity and specificity determination. Positive samples of biological material were obtained from SynLab laboratories, Czech Republic. Negative samples of biological material were obtained from blood donors, Faculty Hospital Královské Vinohrady, Czech Republic.

Table 6

Results of using of AmpliSens® *HCV*-Monitor-L PCR kit

Test material	Number of samples		Negative result	Positive result
Blood plasma	with **true-positive** results of testing	111	0	111
	with **true-negative** results of testing	108	108	0

These samples were also tested with HCV Real-TM Quant Dx PCR kit (Sacace Biotechnologies Srl, Italy).

Table 7

Results of using of HCV Real-TM Quant Dx PCR kit

Test material	Number of samples		Negative result	Positive result
Blood plasma	with **true-positive** results of testing	111	4	107
	with **true-negative** results of testing	107*	107	0

*1 sample was excluded from analysis because of non-valid result.

Table 8

Diagnostic characteristics of AmpliSens® *HCV*-Monitor-L PCR kit

Test material	Diagnostic sensitivity (with a confidence level of 95 %)	Diagnostic specificity (with a confidence level of 95 %)
Blood plasma	100 (96.7-100) %	100 (96.6-100)%

14. REFERENCES

1. Handbook "Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics", developed by Federal State Institute of Science "Central Research Institute of Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being.

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® *HCV*-Monitor-L** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
07.02.22 EM	11. Transportation	The clarification "for no longer than 5 days" was deleted
16.03.22 EM	Through the text	Information about ePure Viral Nucleic Acid Extraction Kit (Ecoli Dx, s.r.o., Czech republic) using ePure automated system (Ecoli Dx, s.r.o.) was deleted
	13.3. Diagnostic characteristics	The subsection was added
14.04.22 EM	13.3. Diagnostic characteristics	The sources of the obtained positive and negative samples were specified
27.04.22 EM	Intended use	The intended use of the PCR kit was corrected

AmpliSens®

EC REP

Ecoli Dx, s.r.o., Purkyňova 74/2
110 00 Praha 1, Czech Republic
Tel.: +420 325 209 912
Cell: +420 739 802 523

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