ECOLI

eSens

CE

IVD

For Professional Use Only

eSens HCV genotype QL PCR kit

REF ES3122B

Instructions for Use

1 INTENDED USE

eSens HCV genotype QL PCR kit is an *in vitro* nucleic acid amplification test for differentiation of hepatitis C virus (HCV) genotypes in clinical material (peripheral blood plasma, serum) using real-time hybridization-fluorescence detection of amplified products.

eSens HCV genotype QL PCR kit is designed for detection of HCV genotypes 1a, 1b, 2, 3a, 4, 5a and 6. **eSens HCV genotype QL PCR kit** is recommended for use after detection of hepatitis C virus RNA using quantitative or qualitative Real Time PCR kits.

eSens HCV genotype QL PCR kit can be used in clinical and diagnostic laboratories of medical institutions and research practice. It is necessary to apply the kit only as directed in this user manual.

The results of the PCR analysis are taken into account in the comprehensive diagnosis of diseases. The kit is intended for use by qualified personnel.

The following table lists the HCV subtypes detected by the kit:

***HCV* genotype**	**Detected *HCV* subtypes**
1	1a, 1b
2	2a, 2b, 2c, 2f, 2k
3	3a
4	4a, 4c
5	5a
6	6a, 6b, 6f, 6j, 6m, 6n

2 METHOD

The implemented PCR method is based on the amplification of the target DNA sequence. Detection of HCV genotypes 1a, 1b, 2, 3a, 4, 5a and 6 by polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. In real-time PCR, the

amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during PCR. The real-time monitoring of fluorescence intensity during RT-PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

eSens HCV genotype QL PCR kit uses a "hot-start", which greatly reduces the frequency of non-specific 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 minutes.

HCV genotype detection includes:

(a) Total RNA extraction from the sample simultaneously with the recombinant Internal Control STI-248-rec (IC) sample.

(b) Reverse transcription of cDNA from RNA template.

(c) RT-PCR of HCV cDNA.

To eliminate possible false negative results, the Internal Control STI-248-rec (IC) is included in the assay. This allows to monitor all stages of the analysis and reveal the possible effect of PCR inhibitors on the result.

The PCR kit contains a system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate (dUTP).

Detection of HCV genotypes in a single clinical sample is carried out in several tubes. Either two HCV genotypes or HCV genotype and IC can be distinguished in one tube during the run.

The PCR kit is designed for the Real-Time PCR cyclers with two and more fluorescence detection channels. The table 1 below lists the channels for detection of HCV genotypes for each of the reaction mixtures used:

Table 1

Reaction mixture	**1b/3a**	**1a/2**	**IC/4**	**5a/6**
Optical channel	**Detected *HCV* genotypes**			
FAM	**1b**	**1a**	**IC**	**5a**
JOE	**3a**	**2**	**4**	**6**

3 CONTENT

eSens HCV genotype QL PCR kit contains the components listed in Table 2 and Table 3.

Table 2

Contents of the kit

Reagents	Description	Volume ml	Quantity
PCR-mix-1 *HCV* genotypes 1b/3a	clear liquid from colorless to lilac	0.6	1 tube
PCR-mix-1 *HCV* genotypes 1a/2	clear liquid from colorless to lilac	0.6	1 tube
PCR-mix-1 *HCV* IC/genotype 4	clear liquid from colorless to lilac	0.6	1 tube
PCR-mix-1 *HCV* genotypes 5a/6	clear liquid from colorless to lilac	0.6	1 tube
PCR-buffer-C	colorless clear liquid	0.3	4 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	4 tubes
Positive control of *HCV* cDNA genotypes 1b/3a (C+1b/3a)	colorless clear liquid	0.2	1 tube
Positive control of *HCV* cDNA genotypes 1a/2 (C+1a/2)	colorless clear liquid	0.2	1 tube
Positive control of *HCV* cDNA IC/genotype 4 (C+IC/4)	colorless clear liquid	0.2	1 tube
Positive control of *HCV* cDNA genotypes 5a/6 (C+5a/6)	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)	colorless clear liquid	1.2	1 tube
Internal Control STI-248-rec (IC)	colorless clear liquid	0.5	1 tube

eSens HCV genotype QL PCR kit (ES3122B) is intended for 55 tests (220 amplification reactions) including controls.

Table 3

REVERTA-L reagent kit

Reagents	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

4 REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- RNA extraction kit
- Pipettes (adjustable)
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl)
- Tube racks
- Vortex mixer
- Desktop centrifuge with a rotor for 2ml reaction tubes
- PCR box
- RT-PCR instruments (e.g. Rotor-Gene 3000/6000 (Corbett research, Australia); Rotor-Gene Q (QIAGEN, Germany), CFX96 (Bio-Rad, USA), eQuantia 48 (Ecoli Dx, Czech Republic), DTprime ("DNA-Technology", Russia)
- Disposable polypropylene tubes:
    - thin-walled 0.2ml PCR tubes with flat caps or strips of four 0.1ml Rotor-Gene PCR tubes if a rotor-type instrument is used;
    - thin-walled 0,2 ml PCR tubes with optical transparent domed caps or strips of eight 0,2 ml tubes with optical transparent caps if a plate-type instrument is used;
- Refrigerator for 2-8 °C
- Deep-freezer with a temperature range of minus 24 to minus 16 °C
- Waste container for pipette tips and other disposable consumables
- Disposable powder-free gloves and a laboratory coat

5 TRANSPORT AND STORAGE CONDITIONS

eSens HCV genotype QL PCR kit should be used at a temperature of 20 to 28 °C and relative humidity of 15 to 75%.

Expiry date - 12 months from the date of production.

The kit can be transported at a temperature from 2 °C to 8 °C for a maximum of 7 days.

All components of the **eSens HCV genotype QL PCR kit** should be stored at temperatures from -24 °C to -16 °C for long periods of time when are not in use (with the exception of the Negative control (C-), TE buffer and Internal Control STI-248-rec (IC).

Negative control (C-), TE-buffer, Internal Control STI-248-rec (IC) are stored at 2-8 °C.

All components of PCR-mix-1 should be protected from unnecessary exposure to light.

All components of the kit are stable until the expiration date on the label. The shelf life of reagents before and after first use is the same unless otherwise stated.

6 WARNINGS AND PRECAUTIONS

Only personnel trained in methods of molecular diagnostic and techniques in clinical and diagnostic laboratory are allowed to work with the kit.

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 (samples, controls and amplicons) separately from all other reagents and add it to the reaction mixture in a a distantly separated facility.

Thaw all componentes thoroughly at room temperature before starting the assay.

After thawing, mix the components and centrifuge briefly.

Wear disposable protective gloves and laboratory coats and protect your eyes when handling samples and reagents. Wash your hands thoroughly 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 Instructions Manual were not observed.

Do not use the kit after its expiry date.

Dispose of all specimens and unused reagents in accordance with the local regulations.

Samples should be considered as potentially infectious and handled in biological cabinet in accordance with the 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 vapours, samples and reagents contact with the skin, eyes and mucous membranes. Harmful if swallowed. In case of contact with these solutions, rinse the injured area immediately with water and seek a medical advice if it is necessary.

Safety Data Sheets (SDS) are available on request.

The PCR kit is intended for single use for PCR analysis of a specified number of samples (see section "Content").

The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit only for the intended purpose.

This product should be used only by 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 to the area where the previous step was performed.

7 SAMPLES

eSens HCV genotype QL PCR kit is designed for the analysis of RNA extracted by nucleic acid extraction kits from clinical material (plasma, serum).

Samples can be stored:

- at a temperature of 2-8 °C for up to 3 days;
- at a temperature of minus 68 °C or less for a long time.

8 PROTOCOL

8.1 RNA extraction

Any commercial nucleic acid extraction kit can be used as long as the IVD-CE is validated for the sample types listed.

Ecoli Dx, s.r.o. recommends:

- For automatic extraction

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

RNA extraction of each test sample is carried out in the presence of **Internal Control STI-248-rec (IC).**

Add 10 µl of Internal Control STI-248-rec (IC) to the test and control tubes.

Extract the nucleic acid according to the manufacturer's protocol.

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

Proceed to reverse transcription immediately after RNA extraction.

8.2 Reverse transcription

It is recommended that the following reverse transcription reagent kit REVERTA-L is used:
The total reaction volume is 22 µl, the RNA sample volume is 11 µl.

Preparing tubes

1. Thaw the tubes with RT-mix and RT-G-mix-1 and vortex thoroughly. Remove any drops from the walls of the tubes.
2. Take the required number of 0.2 or 0.5 ml tubes (depends on the type of thermocycler which is used) including a tube for Negative control of extraction (C-). Mark the tubes.

Reverse transcription for 10-12 samples:

a. Prepare the reaction mixture for 12 reactions. To do this, add 5 µl of RT-G-mix-1 to the tube containing the RT mixture and vortex. To remove drops from the tubes walls, centrifuge briefly.
b. Add 6 µl of revertase (MMlv) to the tube with the reaction mixture, pipette 5 times, and vortex. To remove drops from the tubes walls, centrifuge briefly.
c. Reverse transcription for less than 10 samples:

a. In a new tube mix the reagents in the following order: 10 µl RT-mix, 0,4 µl RT-G-mix-1 and 0,5 µl revertase (MMlv) (the quantities are calculated per one reaction; also see Table 4). When adding RT-G-mix-1 and revertase (MMlv), pipette each reagent at least five times. Vortex the mixture and remove drops from the walls of the tubes. Revertase (MMlv) is temperature-sensitive and should not be kept at room temperature! Place the reagent in the freezer immediately after use.

Table 4

Scheme of reaction mixture preparation:

Volume of reagent per one reaction, µl	**10.0**	**0.4**	**0.5**
Number of clinical samples	**RT-mix**	**RT-G-mix-1**	**Revertase (MMlv)**
4	**60**	**2.4**	**3.0**
5	**70**	**2.8**	**3.5**
6	**80**	**3.2**	**4.0**
7	**90**	**3.6**	**4.5**
8	**100**	**4.0**	**5.0**

3. Transfer 11 µl of the prepared mixture into each tube.
4. Add 11 µl of the RNA sample to each tube with the reaction mixture. Mix gently. To remove drops from the tubes walls, Centrifuge briefly.
5. Place the tubes in a cycler and incubate at 37 °C for 30 minutes.

Table 5

Temperature profile for reverse transcription

Step	**Temperature, °C**	**Time**	**Fluorescence signal detection**	**Cycles**
1	37	30 minutes	-	1

6. After reverse transcription, add 22 µl of DNA-buffer to each tube. Use a new tip for each sample. Vortex the tubes carefully. Make sure there are no drops on the walls of the tubes. Obtained cDNA samples can be used for PCR.

Storage of cDNA samples:

- at a temperature of no more than minus 16 °C for 1 week;
- at a temperature of minus 68 °C or less for 1 year

Reagent volumes are calculated for the amount of clinical samples plus 1 control RNA extraction plus 1 additional reaction.

8.3 Preparing the PCR

8.3.1 Preparing the PCR

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

The type of tubes depends on the PCR instrument used for the analysis. Use disposable filter tips to add reagents, cDNA and controls to the tubes.

Prepare the reaction mixture just before PCR analysis. See Table 6 for a scheme of the reaction mixture preparation.

1. Thaw the reagents, vortex the tubes thoroughly, and centrifuge briefly to remove drops from the walls of the tubes.
2. Take the required number of PCR tubes (including 1 control of RNA extraction and 2 controls of amplification).

NOTE: Each sample should be analyzed with the use of 4 reaction mixtures, therefore, prepare 4 tubes for each sample. If a rotor-type instrument is used, label the 0.2 ml tubes as follows:

Sample No. _1b/3a; Sample No. _1a/2; Sample No. _IC/4; Sample No. _5a/6.

If a striped tubes are used for rotor-type instrument or if the analysis is carried out in a plate-type instrument, use a marked plate.

3. Collect 4 tubes of 0.5-1.5 ml for the preparation of reaction mixtures. Label the tubes 1b/3a, 1a/2, IC/4 and 5a/6.
4. In each of the four labeled tubes add the following reagents (calculating per one reaction):
   5 µl PCR-buffer-C
   0.5 µl polymerase (TaqF)
   10 µl of the desired PCR-1- HCV genotype mixture (see Table 6).
   Make sure that PCR-mix-1 HCV genotypes 1b/3a mixture is added to the tubes labelled 1b/3a and so on. Vortex the tubes with the prepared reaction mixtures and centrifuge briefly to remove drops from the tubes walls.

Table 6

Scheme of reaction mixture preparation

Volume per 1rx/µl		10.0	5.00	0.50
Number of clinical samples	Number of tested samples	PCR-mix-1*	PCR-buffer-C*	Polymerase (TaqF)*
4	7	80	40	4.0
5	8	90	45	4.5
6	9	100	50	5.0
7	10	110	55	5.5
8	11	120	60	6.0
9	12	130	65	6.5
10	13	140	70	7.0
11	14	150	75	7.5
12	15	160	80	8.0

* There is one additional mixed reaction include.

5. Transfer 15 µl of the prepared mixture to the PCR tubes as is indicated. Make sure that the PCR-1 HCV genotype 1b/3a mixture is added to the tubes labelled as 'sample no. _1b/3a' and so on.
6. Add 10 µl of cDNA samples obtained by reverse transcription to the appropriate tubes.
7. Carry out control amplification reactions:

NCA-	Add 10 µl of TE buffer to the tubes containing 1b/3a, 1a/2, IC/4 and 5a/6 reaction mixture (negative control of amplification).
C+1b/3	Add 10 µl of positive control cDNA HCV genotypes 1b/3a (C+1b/3a) to the tube containing the 1b/3a reaction mixture (positive control of amplification).
C+1a/2	Add 10 µl of positive control cDNA of HCV genotypes 1a/2 (C+1a/2) to the 1a/2 reaction mixture tube (positive control of amplification).
C+IC/4	Add 10 µl of positive control cDNA HCV IC/genotype 4 (C+IC/4) to the tube containing the IC/4 reaction mixture (positive control of amplification).
C+5a/6	Add 10 µl of positive control cDNA HCV genotypes 5a/6 (C+5a/6) to the tube containing 5a/6 reaction mixture tube (positive control of amplification)

8.3.2 Amplification

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

Table 7

Temperature profile

	Rotor-type instruments (For example, Rotor-Gene Q or equivalent.)			**Plate-type instruments** (For example, Cfx 96 Touch, DT-96 or equivalent.)		
Step	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
1	95	15 minutes	1	95	15 minutes	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 detection FAM, JOE		60	30 s Fluorescence detection FAM, JOE	
	72	15 s		72	15 s	

The fluorescence signal is detected in the channels for the FAM and JOE fluorophore.

2. Insert the tubes into the reaction module of the device.
3. Run the amplification program with fluorescence detection.
4. Analyze the results after the amplification program is completed.

8.4 Real-Time instrument settings

Settings for Real-Time Rotor-Type instruments

Channel	Calibrate/Gain Optimisation	Threshold value	"Dynamic tube"	"Slope correct."	More Settings / Outlier Removal
FAM/Green	from 5 FI to 10 FI	0,03	ON	ON	8%
JOE/yellow	from 5 FI to 10 FI	0,035	ON	ON	8%

Settings for Real-Time Plate-Type instruments

Set the heating/cooling ***"Ramp rate" to* 2.5 °C/s**.

Channel	Threshold value
FAM, JOE/HEX	Set the threshold line for each channel on **a logarithmic scale** at the level of 10-20 % of the maximum fluorescence obtained for the positive control (C+) in the last amplification cycle.

9 DATA ANALYSIS

Analysis of the results is performed by the software of the RT-PCR instrument used. Curves of accumulation of fluorescent signal in the channel for FAM and JOE fluorophore are analysed.

The results are interpreted by the crossing (or not crossing) of the fluorescence curve with the threshold line set at a specific level. That determines presence (or absence) of Ct value (cycle threshold) of the sample in the appropriate cell of the result grid.

The result of amplification in the channel is considered positive if the fluorescence curve is S-shaped and crosses the threshold line in the area of reliable growth of fluorescence. The result of amplification in the channel is considered negative if the fluorescence curve does not have the typical shape and does not cross the threshold line (Ct or Cp value is undefined). In all other cases, the result of amplification in the channel is considered equivocal. Table 8 lists the channels for detection of HCV genotypes for each of the reaction mixtures used in the PCR assay.

Table 8

HCV genotype detection channels for each of the reaction mixtures

Reaction mixture	1b/3a	1a/2	IC/4	5a/6
Channel for fluorophore	Detected *HCV* genotype			
FAM	1b	1a	IC	5a
JOE	3a	2	4	6

9.1 Interpretation of results for control samples

The result of the analysis is considered reliable only if the results obtained for the positive and negative controls of amplification and for the negative control of extraction are correct (see Table 9).

Table 9

Evaluation table

PCR mix	1b/3a		1a/2		IC/4		5a/6	
Control	Result of amplification in the channel for fluorophore							
	FAM	**JOE**	**FAM**	**JOE**	**FAM**	**JOE**	**FAM**	**JOE**
C-	*Ct* is absent	*Ct* is absent	*Ct* is absent	*Ct* is absent	**<boundary *Ct* value**	*Ct* is absent	*Ct* is absent	*Ct* is absent
NCA	*Ct* is absent	*Ct* is absent	*Ct* is absent	*Ct* is absent	*Ct* is absent	*Ct* is absent	*Ct* is absent	*Ct* is absent
C+1b/3a	**<boundary *Ct* value**	**<boundary *Ct* value**	*	*	*	*	*	*
C+1a/2	*	*	**<boundary *Ct* value**	**<boundary *Ct* value**	*	*	*	*
C+IC/4	*	*	*	*	**<boundary *Ct* value**	**<boundary *Ct* value**	*	*
C+5a/6	*	*	*	*	*	*	**<boundary *Ct* value**	**<boundary *Ct* value**

* Not analyzed with the indicated reaction mixture.

NOTE: Boundary Ct values are specified in Table 10.

Interpretation of results for clinical samples

1. The HCV genotype is determined by comparison of amplification results obtained from the four reaction tubes according to Table 8.

   Take into account the following:

   - If the detected Ct value represents a single HCV genotype, then the result "Genotype..." is displayed;
   - If two or more Ct values are detected for a sample, the dual, triple, etc. genotype is displayed.

However, there is an exception:

If the Ct value is detected in both channel for JOE fluorophore for the IC/4 reaction mixture (HCV genotype 4) and the channel for FAM fluorophore for the 1b/3a reaction mixture (HCV genotype 1b) and the Ct value of HCV genotype 4 is less than the Ct value of HCV genotype 1b for 10 cycles, the result "Genotype 4" is displayed.

If only the Ct value of the Internal Control STI-248-rec (IC) (IC/4 reaction mixture, channel for FAM fluorophore) is detected in the evaluation table and this value is less than the Ct threshold value specified in Table 10, the result "HCV genotype not detected" is displayed. In addition, if the HCV RNA

concentration is known to be within the limits of analytical sensitivity of the PCR kit, then the result 'HCV genotype not detected due to low viral load' should be displayed.

If the Ct values corresponding to all genotypes are absent, while the Ct value for the Internal Control STI-248-rec (IC) (IC/4 reaction mixture, channel for FAM fluorophore) is absent or greater than the boundary Ct value (specified in Table 10), the PCR analysis should be repeated beginning with the RNA extraction phase.

Table 10

***Ct* boundary values**

Sample	**Reaction mixture**	**Rotor-type instrument**		**Plate-type instrument**	
		Channel for fluorophore			
		FAM	**JOE**	**FAM**	**JOE**
C+1b/3a	1b/3a	30	29	34	33
C+1a/2	1a/2	30	29	34	33
C+IC/4	IC/4	30	31	34	35
C+5a/6	5a/6	30	30	34	34
C-	IC/4	31	-	35	-
Test samples	IC/4	31	-	35	-

10 TROUBLESHOOTING

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

1. If the Ct value of at least one positive control of amplification (C+1b/3a, C+1a/2, C+IC/4 or C+5a/6) is greater than the Ct threshold value specified in Table 10 or absent, the PCR analysis should be repeated for all samples beginning from the RNA extraction phase.
2. If a positive signal is detected for the negative control of extraction (C-) with at least one of the following reaction mixtures: 1b/3a, 1a/2, 5a/6 in any channel and/or with the IC/4 reaction mixture in the channel for JOE fluorophore, the PCR analysis should be repeated beginning from the RNA extraction phase for all samples for which the HCV genotype has been detected with this reaction mixture.
3. If a positive signal is detected for the negative control of amplification (NCA) in any of the channels with any reaction mixtures, the PCR analysis should be repeated for all samples in which the HCV genotype has been detected with this reaction mix, beginning from the RNA extraction phase.

11 SPECIFICATIONS

11.1 Sensitivity

Table 11

Extraction volume, µl	Nucleic acid extraction kit	PCR kit	Analytical sensitivity, IU/ml
100	ePure Viral NA Extraction kit	eSens HCV genotype QL PCR kit	$5x10^3$

Analytical specificity

The analytical specificity of the **eSens HCV genotype QL PCR kit** is ensured by the selection of specific primers and probes and by the reaction conditions. The primers and probes were tested for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The absence of cross-reactions between HCV genotypes 1a, 1b, 2, 3a, 4, 5a and 6 was confirmed with the use of highly concentrated recombinant positive control samples and plasma samples as part of the evaluation of the analytical specificity of the PCR kit.

The clinical specificity of the **eSens HCV genotype QL PCR kit** was been confirmed in the clinical trials.

Diagnostic characteristics

265 positive and 102 negative samples of biological material (blood plasma) were used to determine the diagnostic sensitivity and specificity. Positive samples of biological material were obtained from SynLab, Czech Republic. Negative samples of biological material were obtained from blood donors, University Hospital Královské Vinohrady, Czech Republic.

Clinical material was previously tested by HCV NAT (HCV Real-TM Quant Dx kit (Sacace Biotechnologies Srl, Italy)). All results were evaluated as valid for HCV. HCV Genotype Plus (1a,1b,2,3a,4,5a,6) (Sacace Biotechnologies Srl, Italy) was used as a reference assay. The results are listed in Table 13 and 14.

Table 12

Samples type	The results of application of eSens HCV genotype QL PCR kit		Results of using the reference assay	
			Positive	Negative
Blood plasma	367* samples	Positive	265	0
		Negative	0	102

Table 13

Test material	Diagnostic sensitivity* (with a confidence level of 95%)	Diagnostic specificity** (with a confidence level of 95%).
Blood plasma	100 (98.4-100) %	100 (96.4-100)%

* Relative sensitivity in comparison with the applied reference assay.

** Relative specificity in comparison with the applied reference assay.

12 QUALITY CONTROL

Ecoli Dx, s.r.o. declares that the above products comply with the requirements of Council Directive 98/79/EC for *in vitro* diagnostic medical devices. Quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in the production of IVDD products;
- creating value for customers;
- maintaining the best quality of service and customer management.

13 KEY TO THE SYMBOLS USED

	Catalogue number		Contains sufficient quantities for <n> tests
	Batch number		Use-by Date
	*In vitro* diagnostic medical device		Read the instructions for use
	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
	Caution	IC	Internal Control STI-248-rec (IC)

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		
02_08/2024	Through the text	Names of reagents are changed: „PCR-mix-2“ to „PCR-buffer-C“; „Positive *HCV* cDNA control genotypes 1a/2 (C+1a/2)“ to „Positive control of HCV cDNA genotypes 1a/2 (C+1a/2)“; „Positive *HCV* cDNA control IC/genotype 4 (C+IC/4)“ to „Positive control of HCV Cdna IC/genotype 4 (C+IC/4)“; „Positive HCV cDNA control genotypes 5a/6 (C+5a/6)“ to „Positive control of HCV cDNA genotypes 5a/6 (C+5a/6)“; „Internal Control (IC)“ to „Internal Control STI-248-rec (IC)“. REF number is changed: „(EDA0001)“ to „(ES3122B)“.
03_10/2025	9. DATA ANALYSIS	Table 10 is changed.

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