RUO

For Professional Use Only

AmpliSens® *HDV*-Monitor-FRT

PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE

AmpliSens® *HDV*-Monitor-FRT PCR kit is an *in vitro* nucleic acid amplification test for quantitative detection of *hepatitis D virus* (*HDV)* RNA in biological materials (blood plasma) using real-time hybridization-fluorescence detection of amplified products.

For research use only. Not for diagnostic procedures.

2. PRINCIPLE OF PCR DETECTION

HDV detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *HDV* primers. In the 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 thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® *HDV*-Monitor-FRT PCR kit is a quantitative test that contains the Internal Control (Internal Control *ICZ*-rec (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. To obtain the complementary DNA (cDNA) on the RNA matrix, a reverse transcription reaction is required.

AmpliSens® *HDV*-Monitor-FRT PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *HDV*-Monitor-FRT PCR kit is produced in 1 form:

Form 1: PCR kit variant FRT, ***HDV-Q* calibration kit**, **REF** R-V3-MC(RG,iQ,Mx,Dt)-CE.

PCR kit variant FRT includes:

Reagent	***Description***	***Volume, ml***	***Quantity***
DTT frozen-dried	white powder	–	4 tubes
RT-PCR-mix-1-FL *HDV*	colorless clear liquid	0.3	4 tubes
RT-PCR-mix-2-FEP/FRT	colorless clear liquid	0.2	4 tubes
Polymerase (TaqF)	colorless clear liquid	0.02	4 tubes
TM-Revertase (MMlv)	colorless clear liquid	0.01	4 tubes

Reagent		*Description*	*Volume, ml*	*Quantity*
DNA calibrators	**PIC1 *HDV****	colorless clear liquid	0.1	4 tubes
	PIC2 *HDV***	colorless clear liquid	0.1	4 tubes
Buffer for elution		colorless clear liquid	1.2	4 tubes
Negative Control (C–)***		colorless clear liquid	1.2	4 tubes
Positive Control-1-*HDV*****		colorless clear liquid	0.06	4 tubes
Positive Control-2-*HDV*****		colorless clear liquid	0.06	4 tubes
Internal Control *ICZ*-rec (IC)*****		colorless clear liquid	0.28	4 tubes

* must be used in the amplification procedure as Positive Control of Amplification ($C+_1$).

** must be used in the amplification procedure as Positive Control of Amplification ($C+_2$).

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

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

***** add the required volume of Internal Control during the RNA extraction procedure directly to the sample/lysis mixture (see section 8.1 for details).

PCR kit variant FRT is intended for 80 reactions, including controls and calibrators.

***HDV-Q* calibration kit** includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
Calibrator *HDV*-Q	yellow powder	–	1 tube
Solvent Q	colorless clear liquid	1.2	3 tubes

PCR kit also includes:

Compact Disk with software (Microsoft® Excel format) for data interpretation and result analysis obtaining.

4. ADDITIONAL REQUIREMENTS

- DNA/RNA extraction kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile RNase-free/DNase-free pipette tips with filters up to 200 µl.
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2 ml reaction tubes.
- PCR box.

- Real-time instruments (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); iCycler iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA) instrument.
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml:
  a) 0.2-ml PCR tubes with optical transparent domed caps if a plate-type instrument is used;
  b) 0.2-ml PCR tubes with flat caps if a rotor-type instrument is used.
- Refrigerator with a temperature range from 2 to 8 °C.
- Deep-freezer with a temperature range from minus 16 to minus 24 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.

- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

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

6. SAMPLING AND HANDLING

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

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

Blood samples are collected in the morning on an empty stomach 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 taking. Remove obtained plasma and transfer to the new tubes.

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.

The plasma and serum samples can be stored:

– at 2–8 °C for up to 3 days;

– at ≤ – 68 °C for a long time.

7. WORKING CONDITIONS

AmpliSens® *HDV*-Monitor-FRT PCR kit should be used at 18–25 °C

8. PROTOCOL

8.1. RNA extraction

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

- **RIBO-prep**, **REF** K2-9-Et-100-CE
- **MAGNO-sorb**, **REF** K2-16-1000-CE
- **NucliSENS easyMAG** automated system (for details see Guidelines [2]).

<u>If using RIBO-prep kit</u> extract the RNA/DNA according to the manufacturer's protocol taking into account next additions and improvements :

- If a large number of samples is being tested, it is acceptable to mix the **Solution for Lysis** and the **Internal Control *ICZ*-rec (IC)** in a separate sterile flask (based on addition of **300 µl** of **Solution for Lysis** and **10 µl** of **Internal Control *ICZ*-rec (IC)** per one sample), followed by a transfer of **300 µl** of the prepared mix into each of the previously prepared **1.5 ml** tubes.
- For each panel it is necessary to carry out **two** positive controls of extraction – PCE-1 and PCE-2. To the prepared tube containing **Solution for Lysis** and labelled PCE-1 add **90 µl** of **Negative Control (C–)** and **10 µl** of **Positive Control-1-*HDV***, to the tube labelled PCE-2 add **90 µl** of **Negative Control (C–)** and **10 ml** of **Positive Control-2-*HDV***. Vortex the tubes and sediment the drops from the cap.
- Centrifuge at 12,000 g throughout the extraction procedure.

<u>If using the MAGNO-sorb kit</u> extract RNA/DNA according to the manufacturer's protocol taking into account following additions and improvements:

- In case of RNA extraction from blood plasma sample of 1000 µl, the volume of the **Internal Control *ICZ*-rec (IC)** required for 24-tube panel is **0.28 ml**. In case of other panels and DNA extraction from blood plasma sample of 200 µl see the MAGNO-sorb instruction manual.
- For each panel it is necessary to carry out **two** positive controls of extraction – PCE-1 and PCE-2. To the prepared tube containing **Lysis Solution MAGNO-sorb** and labelled PCE-1 add **90 µl** of **Negative Control (C–)** and **10 µl** of **Positive Control-1-*HDV***, to the tube labelled PCE-2 add **90 µl** of **Negative Control (C–)** and **10 µl** of **Positive Control-2-*HDV***. Vortex the tubes and sediment the drops from the cap.
- To prepare the Negative Control of Extraction (**C–**), add **100 µl** of the **Negative Control (C–)** sample to the new tube containing **Lysis Solution MAGNO-sorb**.
- The volume of **Buffer for elution** required for extraction from both 1000 and 200 µl of blood plasma samples is **70 µl**.

8.1.1 Calibration and calculation of the coefficient B using *HDV-Q* calibration kit

If coefficient B for the extraction kit/automatic platform is not specified in the *Important Product Information Bulletin* enclosed in the PCR kit, the calibration for calculation of coefficient B should be carried out by oneself with the aid of the ***HDV-Q* calibration kit** included in this PCR kit. See below for details.

Calibration is necessary for coefficient B detection and performed during the <u>first</u> PCR run for each new PCR kit lot. Calibration is performed <u>only once for each new lot</u> of **AmpliSens® *HDV*-Monitor-FRT** PCR kit and is conducted using the same RNA extraction kit/automatic station as is used in the PCR assay.

To carry out calibration, it is necessary to analyze 5 extra samples during the first run of the given lot: the additional repeat of Positive Control-1-*HDV*, the additional repeat of Positive Control-2-*HDV*, and calibrator *HDV*-Q in triplicate.

Calibrator *HDV*-Q preparation

1. Vortex the tube with calibrator *HDV*-Q, gently open the tube, and add **400 µl of solvent Q** avoiding contents spraying. Use tips with filters.
2. Close the tube and incubate it at room temperature for 20 min. Vortex periodically while incubating.
3. By the end of dissolution, vortex the tube for 3 – 5 sec to make sure that there are no drops on the walls of the tube.

Perform calibration with the same RNA extraction kit used in the PCR assay. Extract according to the manual provided by the manufacturer. Transfer 10 µl of Internal Control ICZ-rec (IC) (per one sample) to samples or to **Lysis solution** before extraction.

In case of extraction from 100 µl of plasma, add dissolved calibrator *HDV*-Q to three tubes for RNA extraction (100 µl per each tube).

In case of extraction from any other plasma volume (100 – 1000 µl), transfer dissolved calibrator *HDV*-Q to three tubes for RNA extraction (100 µl per each tube) and add solvent Q up to the extraction volume (for example, if volume of extraction is 1 ml then add 100 µl of calibrator *HDV*-Q and 900 µl of solvent Q).

When extraction is completed, perform RT-PCR as described in this Instruction Manual.

Use the mean concentration values obtained in the channels for the FAM and JOE fluorophores for three repeats with **calibrator *HDV*-Q** for calculation of coefficient B using the following formula:

$$\textbf{Coefficient B} = \frac{\text{IC cDNA copies in calibrator } \textit{HDV}\text{-Q (FAM channel)}}{\textit{HDV} \text{ cDNA copies in calibrator } \textit{HDV}\text{-Q (JOE/HEX channel)}} \times \text{coefficient C}$$

Coefficient C is specified in the *Important Product Information Bulletin* enclosed in the AmpliSens® *HDV*-Monitor-FRT PCR kit.

The calculated value of coefficient B should be within range specified in the *Important Product Information Bulletin* enclosed in the applied PCR kit lot.

Write down the coefficient B value in the *Important Product Information Bulletin* enclosed in the given lot of the PCR kit and use it for calculation of biological and control sample concentrations.

Write down the calculated values for Positive Control-1-*HDV* and Positive Control-2-*HDV* in the *Important product information bulletin* enclosed in the given lot of the PCR kit.

Determine the mean value for both Positive Control-1-*HDV* and for Positive Control-2-*HDV*. Set the acceptable value range for both Positive Control-1-*HDV* and for Positive Control-2-*HDV* as follows: from *"calculated mean value" / 3* to *"calculated mean value" x 3.*

For example,

the calculated values for Positive Control-1-*HDV* in two repeats are 500,000 IU/ml and 700,000 IU/ml;

the calculated mean value for Positive Control-1-*HDV* is 600,000 IU /ml;

the acceptable value range for Positive Control-1-*HDV* varies from 200,000 to 1,800,000 IU/ml.

8.2. Preparing reverse transcription and PCR

Total reaction volume is **50 µl**, the volume of RNA sample is **25 µl**.

8.2.1 Preparing tubes

All components of the reaction mix should be mixed just before use.
See Table 1 for the reaction mixture preparation scheme.

1. Thaw all reagents, thoroughly vortex, and make sure that there are no drops on the walls of the tubes.
2. Collect the required number of the PCR tubes for amplification of biological and control samples (including 3 controls of extraction and 4 calibrators).
3. To prepare the **reaction mixture:**
   - add the entire contents of the tube with **RT-PCR-mix-2-FEP/FRT** to the tube with **DTT dried-frozen**. Thoroughly vortex and make sure there are no drops on the walls of the tube. Store the prepared mixture at 2–8 °C for no longer than 2 weeks.
   - take a new tube and mix the following reagents calculating per one reaction: **15 µl of RT-PCR-mix-1-FL *HDV***, **10 µl of the mixture of RT-PCR-mix-2-FEP/FRT and DTT frozen-dried, 1.0 µl of polymerase (TaqF) and 0.5 µl of TM-Revertase (MMlv).** Vortex thoroughly and make sure that there are no drops on the walls of the tubes.

It is recommended that the reaction mixture for 20 reactions is prepared in case of extraction from 12 to 16 samples (two NucliSENS easyMAG arrays). To do this, into the tube with **DTT frozen-dried** transfer the entire contents of the tube with **RT-PCR-mix-2-FEP/FRT, RT-PCR-mix-1-FL *HDV*, polymerase (TaqF), and TM-Revertase (MMlv)**. Do not store the prepared mixture!

Table 1

Scheme of reaction mixture preparation

			Reagent volumes per number of samples specified plus 1 extra reaction			
Reagent volume per one reaction, µl			**15.00**	**10.00**	**1.00**	**0.5**
Number of biological samples	Number of extracted samples[1]	Number of samples analyzed in PCR [2]	RT-PCR-mix-1-FL *HDV*	Mixture of RT-PCR-mix-2-FEP/FRT and DTT frozen dried	Polymerase (TaqF)	TM-revertase (MMlv)
3	**6**	**10**	**165**	**110**	**11**	**5.5**
4	**7**	**11**	**180**	**120**	**12**	**6**
5	**8[3]**	**12**	**195**	**130**	**13**	**6.5**
6	**9**	**13**	**210**	**140**	**14**	**7**
7	**10**	**14**	**225**	**150**	**15**	**7.5**
8	**11**	**15**	**240**	**160**	**16**	**8**
9	**12[4]**	**16**	**255**	**170**	**17**	**8.5**
10	**13**	**17**	**270**	**180**	**18**	**9**
11	**14**	**18**	**285**	**190**	**19**	**9.5**
12	**15**	**19**	**Entire contents of the tube**	**Entire contents of the tube**	**Entire contents of the tube**	**Entire contents of the tube**
13	**16[5]**	**20**	**Entire contents of the tube**	**Entire contents of the tube**	**Entire contents of the tube**	**Entire contents of the tube**

4. Transfer 25 µl of the prepared mixture per each PCR tube. Discard unused reaction mixture.
5. Using tips with filter add **25 µl** of biological **RNA samples**. Thoroughly mix by pipetting. Avoid air bubbling.

Avoid transferring of sorbent together with the RNA sample in case of extraction with NucliSENS easyMAG automated system.

6. Carry out the control amplification reactions:

PCE 1 – Add **25 µl of RNA sample** extracted from Positive Control-1-*HDV* to the tube labelled PCE-1;

PCE 2 – Add **25 µl of RNA sample** extracted from Positive Control-2-*HDV* to the tube labelled PCE-2;

C– – Add **25 µl of RNA sample** extracted from Negative Control (C–) to the tube labelled C–;

$C+_1$ – Add **PIC1 *HDV*** to the two tubes labelled $C+_1$ (**25 µl per each tube)**;

$C+_2$ – Add **PIC2 *HDV*** to the two tubes labelled $C+_2$ (**25 µl per each tube)**.

[1] Number of biological samples + 3 controls of RNA extraction (N+3, N – number of biological samples).
[2] Number of biological samples + 3 controls of RNA extraction + 4 DNA calibrators (N+7, N – number of biological samples).
[3] Extraction of 1 stripe with NucliSENS easyMAG automated system (8 tubes).
[4] 12-tube extraction panel.
[5] Extraction of 2 stripes with NucliSENS easyMAG automated system (16 tubes).

Thoroughly mix by pipetting. Avoid air bubbling.

To rule out possible contamination, run an additional control reaction:

NCA – Add **25 µl** of **Buffer for elution** to the tube labelled NCA (Negative Control of Amplification).

8.2.2 Reverse transcription and amplification

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

Table 2

AmpliSens-2 RG program (for rotor-type instruments[6])

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*
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	20 s	FAM, JOE, ROX, Cy5	
	72	15 s	–	

Table 3

AmpliSens-2 iQ program (for plate-type instruments[7])

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*
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, ROX, Cy5	
	72	15 s	–	

The use of either **AmpliSens-2 RG** or **AmpliSens-2 iQ** programs allows to simultaneously carry out any test combinations just in one run (for example simultaneously with *HBV* detection tests; *HCV* genotyping and others).

Channels for the ROX and Cy5 fluorophores are activated upon request, when the multiprime-format tests are carried out, which use these channels.

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

[6] For example, Rotor-Gene 3000/Rotor-Gene 6000 (Corbett Research, Australia).
[7] For example, iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA).

9. DATA ANALYSIS

Results interpretation

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 cDNA amplification product is detected in the channel for the FAM fluorophore.
– The signal of the *HDV* cDNA amplification product is detected in the channel for the JOE fluorophore.

The results are interpreted by the crossing (or not crossing) of the fluorescence curve with the threshold line set at the specific level (in the middle of the linear fragment of the positive control fluorescence growth in the log scale), that corresponds to the presence (or absence) of *Ct* values of the cDNA sample in the corresponding column of the results grid.

Based on the *Ct* values (the intercept of the fluorescence curve and the threshold line set at a certain level) and on the specified values for the calibrators PIC1 *HDV* and PIC2 *HDV* the calibration line will automatically plot and produce the values for the number of *HDV* cDNA copies (channel for the JOE fluorophore) and for the number of Internal Control cDNA copies (channel for the FAM fluorophore) in a PCR sample. The obtained values are used for the *HDV* RNA concentration calculation in test and control samples, using the formulae:

$$\frac{\textit{HDV}\text{ cDNA copies per PCR-sample}}{\text{IC cDNA copies per PCR-sample}} \text{ x coefficient A x coefficient B = IU/ml of plasma}$$

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

Coefficient A = 1 when calculating PCE-1 and PCE- 2 concentrations.

Coefficient B is specified in the *Important Product Information Bulletin* enclosed in the PCR kit and specific for each lot. It cannot be used with PCR kits of different lots. Coefficient B should be calculated as the result of calibration during the first PCR run (see section 8.1.1 for details).

If the result is greater than 100,000,000 IU/ml, then it is interpreted as the **greater than 100,000,000 IU/ml result.** If the result is higher than the linear range, the sample may be re-tested after 10x dilution; the produced result is multiplied by10. If the result is less than 40 IU/ml (extraction from 100 µl), less than 20 IU/ml (extraction from 200 µl), or less than 4 IU/ml (extraction from 1 ml), then it is interpreted as the **less than 40, less than 20, or less than 4 IU/ml result**, respectively.

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 Positive and Negative Control of extraction are correct (see Table 4).

Table 4

Results for controls

Control	Stage for control	Result of amplification in the channel for fluorophore	
		FAM	**JOE**
C–	RNA extraction, PCR	Pos (number of copies of IC cDNA in the sample is greater than the boundary value)	Neg
PCE 1	RNA extraction, PCR	Pos (number of copies of IC cDNA in the sample is greater than the boundary value)	**Pos** (should be within range specified in the Bulletin as a result of calculation with IC copies/ml)
PCE 2	RNA extraction, PCR	Pos (number of copies of IC cDNA in the sample is greater than the boundary value)	**Pos** (should be within range specified in the Bulletin as a result of calculation with IC copies/ml)
$C+_1$	PCR	Pos	Pos
$C+_2$	PCR	Pos	Pos
NCA	PCR	Neg	Neg

Boundary values and the range of values for **PCE-1 (Positive Control-1-*HDV)*** and **PCE-2 (Positive Control-2-*HDV)*** calculated with IC copies/ml are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

10. TROUBLESHOOTING

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

1. If the *Ct* value for the Positive Control of Extraction (PCE) or the Positive Control of Amplification (C+) in the channel for the JOE fluorophore is absent, PCR should be repeated from the DNA extraction stage for all samples in which *HDV* RNA is not found.
2. If the *Ct* value is present for the Negative Control of Extraction (C–) in the channel for the JOE fluorophore and/or for the Negative Control of Amplification (NCA) in the channels for the FAM and JOE fluorophores. PCR should be repeated from the RNA extraction stage for all samples in which *HDV* RNA is found.
3. If the correlation coefficient, R^2, is less than 0.98 when the calibration line is plotted. Repeat PCR for all samples.
4. If the calculated concentrations of Positive Control-1 *HDV* and Positive Control-2 *HDV* is not within the range specified in the *Important Product Information Bulletin*. Repeat the test (from the RNA extraction) for all samples.

11. TRANSPORTATION

AmpliSens® *HDV*-Monitor-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **PCR kit variant FRT** and ***HDV-Q* calibration kit** are to be stored at the temperature from minus 24 to minus 16 °C when not in use. All components of the **AmpliSens® *HDV*-Monitor-FRT** PCR kit are stable until the expiration date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

RT-PCR-mix-1-FL *HDV* is to be kept away from light.

Do not repeat freeze-thaw cycles more than twice for Positive Control-1-*HDV,* Positive Control-2-*HDV,* PIC1 *HDV,* PIC2 *HDV,* Internal Control ICZ-rec. Store the above-mentioned reagents at 2–8 °C for up to 6 month after thawing.

13. SPECIFICATIONS

13.1. Sensitivity

The linear measurement range of **AmpliSens® *HDV*-Monitor-FRT** PCR kit is specified in the table below.

Volume of sample for extraction, µl	DNA/RNA extraction kit	Linear measurement range, IU/ml
100	RIBO-prep NucliSENS easyMAG	40 – 100,000,000
200	MAGNO-sorb	20 – 100,000,000
1000	MAGNO-sorb NucliSENS easyMAG	4 – 100,000,000

13.2. Specificity

The analytical specificity of **AmpliSens® *HDV*-Monitor-FRT** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis as well as with genomic DNA/RNA of the following organisms and viruses: hepatitis A virus; hepatitis B virus; hepatitis C virus; human immunodeficiency virus; cytomegalovirus; Epstein-Barr virus; herpes simplex virus types 1 and 2; *varicella-zoster* virus; human herpes virus types 6 and 8; parvovirus B19; tick-borne encephalitis virus; West Nile encephalitis; adenovirus types 2, 3, and 7; *Escherichia coli*; *Staphylococcus aureus; Streptococcus pyogenes;*

Streptococcus agalactiae; and *Homo sapiens*. No cross-reaction was observed for the abovementioned organisms and viruses.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2010.
2. Guidelines to **AmpliSens® *HCV*-Monitor-FRT**, **AmpliSens® *HBV*-Monitor-FRT**, and **AmpliSens® *HDV*-Monitor-FRT** PCR kits for quantitative detection of *hepatitis C virus* (*HCV)* RNA, *hepatitis B virus* (*HBV)* DNA and *hepatitis D (HDV)* RNA in the biological material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

15. QUALITY CONTROL

In compliance with Federal Budget Institution of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *HDV*-Monitor-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	Σ	Sufficient for
RUO	Research use only		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
PCE	Positive control of extraction	**C+**	Positive control of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
22.06.12 BO	Cover Page	IVD-symbol was changed to RUO
	Text	“tips with aerosol barriers” was changed to “tips with filters”
	Sensitivity	Sensitivity for sample 200 µl was added
	8.1. RNA Extraction	Information about MAGNO-sorb was added
30.10.12 Ivl	Through the text	Line range unit was changed from IU/ml to copies/ml
23.06.14 ME	8.1. RNA extraction	The section was rewritten. The use of the EM-plus kit was deleted, with respect to the template and Russian Instruction Manual, section for NucliSENS easyMAG extraction was moved to the Guidelines. Information about calibration and calculation of the coefficient B using *HDV-Q* calibration kit was added from Appendix 2
	8.2. Preparing reverse transcription and PCR	Table 1 was added from Appendix 1
	Text	Corrections in accordance with template and Russian instruction manual
15.07.14 ME	8.1.1 Calibration and calculation of the coefficient B using *HDV-Q* calibration kit	The phrase “Add two extra tubes (for calibrator *HDV*-Q) when collecting tubes for RNA extraction” was deleted
14.03.15 ME	Through the text	Misprints and inaccuracies was corrected
08.05.15 ME	Text	Clinical material was changed to biological
	1. Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” was changed to “For research use only. Not for diagnostic procedures”
	3. Content	Compact Disk with software was added
	5. General precautions	Corrections in accordance with template
	8.1.1 Calibration and calculation of the coefficient B using *HDV-Q* calibration kit	The units of measurement “copies/ml” were changed to “IU/ml”
	9. Data analysis	The units of measurement “copies/ml” were changed to “IU/ml”. The recalculation was made. The unit of measurement for coefficient B was deleted. In the table with results for controls criteria for the FAM channel were changed
	13.1. Sensitivity	The units of measurement “copies/ml” were changed to “IU/ml”. The recalculation was made
	13.2. Specificity	The phrase “The clinical specificity of AmpliSens® *HDV*-Monitor-FRT PCR kit was confirmed in laboratory clinical trials” was deleted
02.11.15 ME	8.2. Preparing reverse transcription and PCR	The shelf life of the mixture of RT-PCR-mix-2-FEP/FRT and DTT dried-frozen was added
03.11.17 PM	3. Content	The content of form 1 was specified