RUO

For Professional Use Only

AmpliSens® *HBV*-Monitor-FRT

PCR kit

Instruction Manual

AmpliSens®

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

TABLE OF CONTENTS

1. INTENDED USE

AmpliSens® *HBV*-Monitor-FRT PCR kit is an *in vitro* nucleic acid amplification test for quantitative detection of hepatitis B virus *(HBV)* DNA in clinical material (blood plasma) using 'real-time' hybridization-fluorescence detection.

The results of PCR analysis are taken into account in complex diagnostics of disease[1].

2. PRINCIPLE OF PCR DETECTION

HBV DNA detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region by using specific 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.

AmpliSens® *HBV*-Monitor-FRT PCR kit is a quantitative test that contains the Internal Control (**Internal Control STI-87 (IC)**). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. To obtain the complementary DNA (cDNA) on the RNA matrix, a reverse transcription reaction is required.

AmpliSens® *HBV*-Monitor-FRT PCR kit uses "hot-start", which greatly reduces frequency of non-specifically primed reactions. "Hot-start" is guaranteed by separation of nucleotides and Taq-polymerase by using chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENTS

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

AmpliSens® *HBV*-Monitor-FRT PCR kit variant FRT, **REF** R-V5-MC(RG,iQ,Mx,Dt)-CE.

AmpliSens® *HBV*-Monitor-FRT PCR kit variant FRT includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *HBV*	colorless clear liquid	0.3	4 tubes
PCR-mix-2-FRT	colorless clear liquid	0.2	4 tubes
Polymerase (TaqF)	colorless clear liquid	0.02	4 tubes

[1] In compliance with the European Union Directive 98/79/EC.

DNA calibrators	**PIC1 *HBV****	colorless clear liquid	0.1	4 tubes
	PIC2 *HBV***	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-*HBV*****		colorless clear liquid	0.06	4 tubes
Positive Control-2-*HBV*****		colorless clear liquid	0.06	4 tubes
Internal Control STI-87 (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 extraction procedure as Negative Control of Extraction.

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

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

AmpliSens® *HBV*-Monitor-FRT PCR kit is intended for 80 reactions, including controls and DNA calibrators.

HBV-Q calibration kit includes:

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

4. ADDITIONAL REQUIREMENTS

- DNA/RNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free/DNase-free pipette tips with filters up to 200 µl and up to 1000 µl.
- Tube racks
- Vortex mixer.
- Disposable flask for 10-20 µl.
- Desktop microcentrifuge up to 12,000 g (suitable for Eppendorf tubes).
- Thermostat with working temperature range from 25 °C to100 °C.
- Vacuum aspirator with flask for removing a supernatant.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); iCycler iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA)).

- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
  a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  b) 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator with a temperature range from 2 °C to 8 °C.
- Deep-freezer with a temperature range from minus 16 °C 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 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 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 samples and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent 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.
- 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 process 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.

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

6. SAMPLING AND HANDLING

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® *HBV*-Monitor-FRT PCR kit is intended for the analysis of the DNA extracted with DNA extraction kits from the clinical material (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 for 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 peripheral blood plasma and the blood serum samples can be stored for no longer than 3 days at a temperature range from 2 to 8 °C or, if stored at a temperature no greater than minus 68 °C, for a long time.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA 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 nucleic acid extraction system (bioMérieux, France) can also be used. (See Guidelines [2] for details).

If using RIBO-prep kit 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** in a separate sterile flask (based on addition of **300 µl** of **Solution for Lysis** and **10 µl** of **Internal Control** per one sample), followed by a transfer of **300 µl** of the prepared mix into each of the previously prepared **1.5 µl** tubes.
- For each panel it is necessary to set up **two** positive controls of extraction – PCE-1 and PCE-2. To the tube labelled PCE-1 add **90 µl** of **Negative Control**

and **10 µl** of **Positive Control-1-*HBV***, to the tube labelled PCE-2 add **90 µl** of **Negative Control** and **10 µl** of **Positive Control-2-*HBV***. Close the lids and vortex the tubes.

- Centrifuge at 12,000 g throughout the extraction procedure.

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

- In case of DNA extraction from blood plasma sample of 1000 µl, the volume of the **Internal Control STI-87** 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.

- To prepare the Positive Control of Extraction 1 (**PCE-1**), add **90 µl** of the **Negative Control (C–)** sample and **10 µl** of the **Positive Control-1-*HBV*** sample to the new tube containing **Lysis Solution MAGNO-sorb**.
- To prepare the Positive Control of Extraction 2 (**PCE-2**), add **90 µl** of the **Negative Control (C–)** sample and **10 µl** of the **Positive Control-2-*HBV*** sample to the new tube containing **Lysis Solution MAGNO-sorb**.
- 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 *HBV-Q* calibration kit.

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

The calibration procedure is necessary to define Coefficient B and it is performed during the first PCR run for the given lot. Calibration is performed only once for each new lot of the **AmpliSens® *HBV*-Monitor-FRT** PCR kit and is conducted using the same DNA extraction kit/automatic station as was used in the PCR assay. To carry out calibration, it is necessary to analyse 5 extra samples: the repeat of Positive Control-1-*HBV*, the repeat of Positive Control-2-*HBV*, and calibrator *HBV*-Q in triplicate.

Calibrator *HBV*-Q preparation

1. Vortex the tube with **calibrator *HBV*-Q**, gently open the tube, and add **400 µl of solvent Q** avoiding the contents spraying. Use tips with filters.
2. Close the tube and incubate it at room temperature for 20 min vortexing periodically.
3. Once the contents are fully dissolved, vortex the tube for 3-5 s to make sure that there are no drops on the walls of the tube.

Perform calibration with the same DNA extraction kit used in the PCR assay.

Extract the DNA according to the manufacturer's protocol.

Transfer 10 µl of **Internal Control STI-87 (IC)** (per one sample) to samples or **Lysis solution** before extraction.

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

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

When extraction is completed, perform 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 *HBV*-Q** for calculation of coefficient B using the following formula:

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

Coefficient C is specified in the *Important Product Information Bulletin* enclosed in the **AmpliSens® *HBV*-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 with the given lot of the PCR kit and use it for concentrations calculation of clinical and control samples (See the Data Analysis section).

Also see the Guidelines [2] to **AmpliSens® *HBV*-Monitor-FRT** PCR kit.

Write down the calculated values for Positive Control-1-*HBV* and Positive Control-2-*HBV* in the *Important Product Information Bulletin* enclosed with the given lot of the PCR kit.

Determine the mean value for both Positive Contorl-1-*HBV* and for Positive Control-2-*HBV*. Set the acceptable value range for both Positive Contorl-1-*HBV* and for Positive Control-2-*HBV* as follows: from *"calculated mean value"/3* to *"calculated mean value" x 3.*
For example,
the calculated values for Positive Contorl-1-*HBV* in two replicates are 500,000 IU/ml and 700,000 IU/ml;
the calculated mean value for Positive Contorl-1-*HBV* is 600,000 IU/ml;
the acceptable value range for Positive Contorl-1-*HBV* varies from 200,000 to 1800,000 IU/ml.

Write down the calculated acceptable value range for Positive Control-1-*HBV* and for Positive Control-2-*HBV* in the *Important Product Information Bulletin,* and use it to verify further assays conducted using this lot of the PCR kit. (See Data Analysis section)

8.2. Preparing PCR

The total reaction volume is **50 µl**, the volume of DNA sample is **25 µl**.

8.2.1 Preparing tubes for PCR

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

1. Thaw all reagents prior to starting work, 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 the amplification of clinical and control samples (including 3 controls of extraction and 4 calibrators).
3. To prepare the **reaction mixture,** take a new tube and mix the following reagents with volumes per one reaction: **15 µl of PCR-mix-1-FL *HBV***, **10 µl of PCR-mix-2-FRT,** and **1.0 µl of polymerase (TaqF).** 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 **PCR-mix-1-FL *HBV*** transfer the entire contents of the tube with **PCR-mix-2-FRT** and the entire contents of the tube with **polymerase (TaqF).** Do not store the prepared mixture!

Table 1

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**
Number of clinical samples	Number of extracted samples[2]	Number of samples analysed in PCR [3]	PCR-mix-1-FL *HBV*	PCR-mix-2-FRT	Polymerase (TaqF)
3	**6**	**10**	**165**	**110**	**11**
4	**7**	**11**	**180**	**120**	**12**
5	**8[4]**	**12**	**195**	**130**	**13**
6	**9**	**13**	**210**	**140**	**14**
7	**10**	**14**	**225**	**150**	**15**
8	**11**	**15**	**240**	**160**	**16**
9	**12[5]**	**16**	**255**	**170**	**17**
10	**13**	**17**	**270**	**180**	**18**
11	**14**	**18**	**285**	**190**	**19**
12	**15**	**19**	**Entire contents of the tube**	**Entire contents of the tube**	**Entire contents of the tube**
13	**16[6]**	**20**	**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 filters add **25 µl** of clinical **DNA-samples**. Thoroughly mix by pipetting. Avoid formation of air bubbles.

Avoid transferring of the sorbent together with the DNA sample in case of extraction with NucliSENS easyMAG automated nucleic acid extraction system or MAGNO-sorb kit.

6. Carry out the control amplification reactions:

PCE-1 -Add **25 µl of DNA sample** extracted from Positive Control-1-*HBV* to the tube labelled PCE-1;

PCE-2 -Add **25 µl of DNA sample** extracted from Positive Control-2-*HBV* to the tube labelled PCE-2;

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

$C+_1$ -Add **PIC1 *HBV*** to two tubes labelled $C+_1$ (**25 µl per each tube)**;

$C+_2$ -Add **PIC2 *HBV*** to two tubes labelled $C+_2$ (**25 µl per each tube)**.

Thoroughly mix by pipetting. Avoid formation of air bubbles.

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

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

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

8.2.2 Amplification

1. Load the tubes into the wells of the instrument's reaction module.
2. Create a temperature profile on your instrument as follows:

Table 2

AmpliSens-2 RG program (for rotor-type instruments)

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, HEX, ROX, Cy5	
	72	15 s	–	

Table 3

AmpliSens-2 iQ program (for plate-type instruments)

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, HEX, 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. In case of one instrument simultaneously performing only the DNA *HBV* extraction tests, it is possible to omit the first step of the program (50 °C, 15 min) to spare time.

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

3. Adjust the fluorescence channel sensitivity according to the Guidelines [2].
4. Run the amplification program with fluorescence detection.
5. Analyse results after the amplification program is completed.

9. DATA ANALYSIS

Interpretation of results

Analysis of results is performed by the software of the real-time PCR instrument used by

measuring the fluorescence signal accumulation in two channels:

- The signal of the Internal Control DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the *HBV* DNA fragment amplification product is detected in the channel for the JOE fluorophore.

The results are interpreted by the presence (or absence) of the intercept between the fluorescence curve and the threshold line set at a certain level (in the middle of the linear fragment of the positive control fluorescence growth in the log scale), which determines the presence (or absence) of the *Ct* values for this sample in the corresponding cell of the results table.

Based on the *Ct* boundary 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 *HBV* and PIC2 *HBV*, the calibration line will automatically plot and produce the values for the number of *HBV* DNA copies (channel for the JOE fluorophore) and for the number of Internal Control DNA copies (channel for the FAM fluorophore) in a PCR sample. The retrieved values are used for the *HBV* DNA concentration calculation in tested and control samples, using the formulae:

$$\frac{\textit{HBV}\text{ DNA copies per PCR sample}}{\text{IC DNA copies per PCR sample}} \times \text{coefficient A} \times \text{coefficient B} = \text{IU } \textit{HBV}\text{ DNA/ml of plasma}$$

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

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

Coefficient B (IC copies/ml of plasma) is specified in the *Important Product Information Bulletin* enclosed in the PCR kit and is specific for each lot. 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 more than 100,000,000 IU/ml, then it is interpreted as the **more than 100,000,000 IU *HBV*/ml result.** If the obtained value is higher than the linear range, then the sample may be re-tested after 10x dilution; the produced result is multiplied by 10.

If the result is less than 150 IU/ml upon extraction from 100 µl, or less than 75 IU/ml upon extraction from 200 µl, or less than 15 IU/ml upon extraction from 1 ml, then it is interpreted as the **less than 150,** or **less than 75,** or **less than**

15 IU *HBV*/ml result, respectively.

The result calculated in IU/ml can be converted into copies/ml by multiplying by 1.7 (1 IU = 1.70 copies/ml, 1 copy = 0.59 IU).

The results are considered reliable if both positive and negative controls of amplification as well as negative and positive controls of extraction are correct with respect to the following table:

Table 4

Results for controls

Control	Stage for control	Result of amplification in the channel for fluorophore	
		FAM	**JOE**
C-	DNA extraction, PCR	*Ct* ≤ specified value (Pos)	Neg
PCE-1	DNA extraction, PCR	*Ct* ≤ specified value (Pos)	**Pos** (concentration calculated with IC copies/ml should be within range)
PCE-2	DNA extraction, PCR	*Ct* ≤ specified (Pos)	**Pos** (concentration calculated with IC copies/ml should be within range)
C+$_1$	PCR	Pos	Pos
C+$_2$	PCR	Pos	Pos
NCA	PCR	Neg	Neg

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

10. TROUBLESHOOTING

The results are not taken into account in the following cases:

1. If the *Ct* value obtained for the Positive Controls of Extraction or Amplification (PCE or C+) in the channel for the JOE fluorophore is absent or is greater than the specified boundary value, then it is necessary to repeat the test (from the DNA extraction stage) for all samples in which ***HBV* DNA** was not found.
2. If the *Ct* value is obtained for the Negative Control of Extraction (C-) and/or Negative Control of Amplification (NCA) in the channel for the JOE fluorophore, then it is necessary to repeat the test (from the DNA extraction stage) for all samples in which ***HBV* DNA** was found.
3. If the correlation coefficient, R^2, is less than 0.98 upon the plotting of the calibration line, then it is necessary to repeat PCR for all samples.

4. If the calculated concentrations of **Positive Control-1-*HBV*** and **Positive Control-2-*HBV*** exceed the range specified in the *Important Product Information Bulletin*, then it is necessary to repeat the test (from the DNA extraction stage) for all samples.

11. TRANSPORTATION

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

12. STABILITY AND STORAGE

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

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

Do not repeat freeze-thaw cycles more than twice for Positive Control-1-*HBV,* Positive Control-2-*HBV,* PIC1 *HBV,* PIC2 *HBV,* Internal Control STI-87. Store the above reagents at a temperature range from 2 °C to 8 °C for up to 6 month after thawing.

13. SPECIFICATIONS

13.1. Sensitivity

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

Table 5

Clinical material	Volume of sample for extraction, µl	DNA/RNA extraction kit	Linear measurement range, IU/ml
Blood plasma	100	RIBO-prep NucliSENS easyMAG	150 – 100,000,000
	200	MAGNO-sorb	75 – 100,000,000
	1,000	MAGNO-sorb NucliSENS easyMAG	15 – 100,000,000

13.2. Specificity

The analytical specificity of **AmpliSens® *HBV*-Monitor-FRT** PCR kit is ensured by the selection of specific primers and probes as well as stringent 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 RNA/DNA of the following organisms and viruses to the reaction: *hepatitis* A virus; *hepatitis* D virus; *hepatitis* C virus; *human immunodeficiency* virus; *cytomegalovirus*; *Epstein-Barr* virus; *herpes simplex* virus types 1 and 2; *chicken pox* virus; *human herpes* virus types 6 and 8; *parvovirus* B19; *tick-borne encephalitis* virus*; West Nile encephalitis* virus; *adenovirus* types 2, 3, and 7; *Escherichia coli*; *Staphylococcus aureus; Streptococcus pyogenes; Streptococcus agalactiae;* and *Homo sapiens*.

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

The clinical specificity of **AmpliSens® *HBV*-Monitor-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 State Institute of Science “Central Research Institute of 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 clinical 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 Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *HBV*-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		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
03.12.10	Sampling and handling	Sentence «Collect blood samples into tubes with 3% EDTA solution (1 : 20) after overnight fasting» is changed into «Blood samples are taken after overnight fasting into the tube with EDTA solution as anticoagulant».
	Through the text	Corrections through the text
		MAGNO-sorb mention was deleted
	Stability and storage	Phrase about keeping away from light of PCR-mix-1-FL *HBV* is added
13.12.10	Content	DNA calibrators PIC1 *HBV*(C+$_1$) *and* PIC2 *HBV*(C+$_2$) are changed to DNA calibrators PIC1 *HBV* and PIC2 *HBV*
23.12.10 RT	Data analysis	Item was corrected in accordance with Russian instruction
	Stability and storage	*HBV*-Q calibration kit storage conditions are added
20.01.11 RT	Cover page	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 open reagents was added
	Key to Symbols Used	The explanation of symbols was corrected
03.07.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
15.09.11 RT	8. PROTOCOL 8.1. DNA extraction	The information about using RIBO-prep kit was added
02.12.11 VV	3.CONTENT	The mentioning about the PCR kit form that does not correspond to the specified catalogue number is deleted
16.03.12 LA	13.1. Sensitivity	Column “Test material” (blood plasma) is added
		The name of DNA/RNA extraction kit is changed from RIBO-sorb to RIBO-sorb-12
20.06.12 LA	Cover page	Symbol IVD was replaced by RUO symbol
	16. Key to symbols used	
	13.1. Sensitivity	Information related to MAGNO-sorb reagent kit was added to the table of analytical sensitivity
	8.1. DNA extraction	Reference number of MAGNO-sorb reagent kit was added: K2-16-1000-CE
		Information about extraction with MAGNO-sorb is added
23.06.14 ME	2. Principle of PCR detection	Paragraph revised in accordance to the Russian Instruction Manual and the template
	4. Additional Requirements	DNase free filter tips were added to the list
		Corrections through the text
	5 General Precautions	Section corrected with respect to the template
	6. Sampling and Handling	Attention box was added about the blood samples storage
		Corrections through the text

	8.1 DNA extraction	Section corrected, 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, Calibration and calculation of coefficient B using *HBV-Q* calibration kit was added from Appendix 2.
	8.2.1 Preparing tubes for PCR	Table 1 was added from Appendix 1
		The names of positive controls of extraction were changed from “PCE 1” and “PCE 2” to “PCE-1” and “PCE-2”
	9. Data analysis	Chapter rewritten
	10. Troubleshooting	Section corrected with respect to the template and Russian Instruction Manual
	13.1 Sensitivity	Table 5, the name of column 1 was changed from “Test material” to “Clinical material”
	Throughout the text	Minor grammar and syntax corrections
		Tables were numbered