AmpliSens® *HBV*-FRT PCR kit

Instruction Manual

RUO

For Professional Use Only

KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Contains sufficient for <n> tests
LOT	Batch code		Use-by Date
RUO	Research use only		Consult instructions for use
VER	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	PCE	Positive Control of Extraction
		IC	Internal control

1. INTENDED USE

AmpliSens® *HBV*-FRT PCR kit is not a medical device. PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of hepatitis B virus (*HBV*) DNA in the biological material using real-time hybridization-fluorescence detection of amplified products. The material for PCR is DNA samples extracted from blood plasma.

Indications and contra-indications for use of the reagent kit

The reagent kit is used for the analysis of biological material in order to detect hepatitis B. There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

NOTE: For research use only. Not for diagnostic procedures.

2. PRINCIPLE OF PCR DETECTION

Principle of testing is based on the DNA extraction from the samples of test material with the exogenous internal control sample (Internal Control STI-87-rec (IC)) and simultaneous amplification of DNA fragments of the detected virus and DNA of the internal control with hybridization-fluorescence detection. Exogenous internal control (Internal Control STI-87-rec (IC)) allows to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition.

Amplification of DNA fragment is performed with the use of specific primers and Taq-polymerase enzyme. 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® *HBV*-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. The "hot-start" is guaranteed by separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF). The latter is activated by heating at 95 ºC for 15 min.

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

In the PCR step, two DNA-targets are amplified simultaneously in the same tube. The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	IC DNA	*HBV* DNA
Target gene	Artificially synthesized sequence	gene C

3. CONTENT

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

variant FRT REF R-V5-Mod(RG,iQ,Mx,Dt)-CE.

Variant FRT includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL *HBV*	clear liquid from colorless to light lilac colour	0.3	4 tubes
PCR-buffer-B	colorless clear liquid	0.2	4 tubes
Polymerase (TaqF)	colorless clear liquid	0.02	4 tubes
DNA calibrator PIC2 *HBV**	colorless clear liquid	0.1	4 tubes
Buffer for elution	colorless clear liquid	1.2	2 tubes
Negative Control (C–)**	colorless clear liquid	1.2	4 tubes
Positive Control-1-*HBV****	colorless clear liquid	0.06	4 tubes
Internal Control STI-87 (IC)****	colorless clear liquid	0.28	4 tubes

* serves as a Positive Control of Amplification (C+).
** must be used in the extraction procedure as Negative Control of Extraction.
*** must be used in the extraction procedure as Positive Control of Extraction.
****must be added during the RNA/DNA extraction procedure directly to the sample/lysis mixture.

Variant FRT is intended for 112 amplification reactions (including controls).

4. ADDITIONAL REQUIREMENTS

For sampling

- Vacuum tubes for sampling, storage and transportation of blood samples.
- Medical centrifuge with accessories.
- Disposable tightly closed polypropylene 2.0-ml tubes.

For RNA extraction, reverse transcription and amplification

- DNA extraction kit.
- Pipettes (adjustable).
- Sterile RNase/DNase-free pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene tubes:
  a) screwed or tightly closed 1.5-ml tubes for reaction mixture preparation.
  b) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
  c) 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.
- Refrigerator at 2 to 8 °C.
- Deep-freezer at 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

AmpliSens® *HBV*-FRT PCR kit is intended for analysis of DNA extracted with a DNA extraction kits from blood plasma.

Sampling

Blood samples are taken after overnight fasting or in 3 hours after a meal by a disposable 0.8-1.1 mm diameter needle into a tube (special vacuum system) with 6% EDTA solution (K_2EDTA or K_3EDTA) or sodium citrate solution as an anticoagulant. Closed tubes with blood are turned several times upside down and back again to ensure that the blood in the tube is thoroughly mixed with the anticoagulant (otherwise, the blood will clot and DNA extraction will not be possible!), and stored at a temperature of 2 to 8 ° C for no more than 6 hours. Tubes with blood are centrifuged at 800–1600 g for 20 min at room temperature. The obtained plasma is transferred in an amount of at least 1 ml by separate tips with filter into sterile Eppendorf tubes with a volume of 2.0 ml.

Blood plasma samples can be stored before the PCR-analysis:

- 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 not more than minus 68 °C – for a long time.

Only one freeze-thawing cycle is required.

Blood plasma can be transported at a temperature from 2 to 8 °C for no more than 3 days.

In some cases, blood serum can be used. The diagnostic sensitivity may be significantly decreased as a result of precipitation of viral particles during blood clot retraction. Blood serum can be stored at the temperature from 2 to 8 °C for at most 3 days or at the temperature not more than minus 68 °C for a long time.

Pretreatment

Pretreatment of *blood plasma* is not required.

Interfering substances and limitations of using test material samples

In order to control the RNA extraction efficiency and possible reaction inhibition the Internal Control (Internal Control STI-87 (IC)) is used in the PCR kit. The Internal Control is added in each biological sample at the extraction stage. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.

The next samples are inapplicable for analysis:

- blood plasma samples with hemolysis;
- blood plasma samples after repeated freezing-thawing;
- blood plasma samples, obtained from blood samples collected in the tubes with heparin as anticoagulant.

Potential interfering substances

Endogenous and exogenous substances that may be present in the biological material used for the study were selected to assess potential interference (see Table 2).

Model samples of blood plasma without adding and with the addition of potential interfering substances were tested. The concentration of each potential interfering substance is specified in Table 2. Model samples contained quality control samples (QCS) with *HBV* DNA in concentration 500 IU/ml.

Table 2

Type of potential interferent	Potential interferent	Tested concentration in a sample	Interference presence
Endogenous substances	Hemoglobin	160 g/l (upper limit of normal)	Not detected
		250 g/l	
	Triglycerides	3,7 mmol/l (upper limit of normal)	Not detected
		37 mmol/l	
	Bilirubin	21 µmol/l (upper limit of normal)	Not detected
		210 µmol/l	
	Protein	85 g/l (upper limit of normal)	Not detected
		120 g/l	
Exogenous substances	Lithium heparin	12 IU/ml	Detected
	Potassium EDTA	2.0 µg/ml	Not detected

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA extraction

It's recommended to use the following nucleic acid extraction kits:

- **RIBO-sorb**,
- **RIBO-prep**,
- **MAGNO-sorb**.

The DNA extraction of each test and control sample is carried out in the presence of **Internal Control STI-87 (IC)**.

NOTE: **If using RIBO-sorb kit,** extract the RNA according to the manufacturer's protocol taking into account next additions and improvements:

- Add **10 µl** of **Internal Control STI-87 (IC)** to each tube and then add **450 µl** of **Lysis Solution**.
- For a large number of samples, to facilitate the extraction procedure, it is allowed to mix **Lysis Solution** and **Internal Control ICZ-rec (IC)** (450 µl **Lysis Solution** and 10 µl **Internal Control ICZ-rec (IC)** per sample) and then add **450** µl of mixture into each prepared 1.5-ml tubes.
- It is necessary to carry out the positive and negative controls of extraction. To the tube labelled PCE add **90 µl** of **Negative Control (C–)** and **10 µl** of **Positive Control-1-*HBV***. To the tube labelled C– add **100 µl** of **Negative Control (C–).** Close the tubes. Vortex thoroughly and sediment the drops from the caps of the tubes by short centrifugation
- After addition of biological and control samples to **Lysis Solution** warm the mixture at 60 °C for 10 min prior to sorbent addition. Sediment the drops from the caps of the tubes by short centrifugation.

NOTE: **If using RIBO-prep kit**, extract the RNA according to the manufacturer's protocol:

The volumes of reagents and samples when the RNA is extracted by the RIBO-prep reagent kit:

- Add **10 µl** of the **Internal Control STI-87 (IC)** into each tube.
- The volume of the test sample is **100 µl**
- Add **100 µl** of **Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction)
- Add **90 µl** of **Negative Control (C–)** and **10 µl** of **Positive Control-1-*HBV*** to the tube labelled PCE (Positive Control of Extraction).
- The volume of elution is **50 µl.**

NOTE: **If using the MAGNO-sorb kit** extract the RNA according to the manufacturer's protocol

The volumes of reagents and samples when the RNA is extracted by the MAGNO-sorb reagent kit:

- Add **10 µl** of the **Internal Control STI-87 (IC)** into each tube.
- The volume of the test sample:
  - 200 µl;
  - 1000 µl.
- Add **100 µl** of **Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction).
- Add **90 µl** of **Negative Control (C–)** and **10 µl** of **Positive Control-1-*HBV*** to the tube labelled PCE (Positive Control of Extraction).
- The volume of elution is **70 µl**.

8.2. Preparing the PCR

8.2.1 Preparing tubes for PCR

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

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

1. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:
   - **10 µl** of **PCR-mix-1-FL *HBV***,
   - **5 µl** of **PCR-buffer-B**,
   - **0.5 µl** of **Polymerase (TaqF)**.

Prepare the reaction mixture for the total number of test and control samples plus some extra reaction. See numbers of control samples in item 7.

The calculation for the required number of reactions can be performed according to Table 3.

Table 3

Scheme of reaction mixture preparation

		Reagent volume for specified number of reactions plus one extra reaction		
Reagent volume for one reaction, µl		**10.00**	**5.00**	**0.50**
Number of test samples	Number of PCR reactions[1]	PCR-mix-1-FL *HBV*	PCR-buffer-B	Polymerase (TaqF)
4	**7**	**80**	**40**	**4.0**
6	**9**	**100**	**50**	**5.0**
8	**11**	**120**	**60**	**6.0**
10[2]	**13**	**140**	**70**	**7.0**
12	**15**	**160**	**80**	**8.0**
14	**17**	**180**	**90**	**9.0**
16	**19**	**200**	**100**	**10.0**
18	**21**	**220**	**110**	**11.0**
20	**23**	**240**	**120**	**12.0**
22[3]	**25**	**260**	**130**	**13.0**
34	**37**	**380**	**190**	**19.0**
46	**49**	**500**	**250**	**25.0**

NOTE: Prepare the reaction mixture just before use.

2. Thaw all the tubes of PCR kit, thoroughly vortex them and sediment the drops by short centrifugation.
3. Prepare the reaction mixture in a new tube. Mix the required quantities of **PCR-mix-1-FL *HBV*, PCR-buffer-B** and **Polymerase (TaqF)**. Vortex thoroughly and sediment the drops from the caps of the tubes by short centrifugation. Do not store the prepared mixture.
4. Take the required number of the tubes for PCR of test and control samples (see Table 3).
5. Add **15 µl** of the prepared reaction mixture to each PCR tube. Discard the unused reaction mixture.
6. Add **10 µl** of **DNA samples** extracted from the biological samples to each PCR tube.

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

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

7. Carry out the **control reactions**:

PCE – Add **10 µl** of the **DNA sample** extracted from the Positive Control-1-*HBV* to the tube labeled PCE (Positive Control of Extraction).

C- – Add **10 µl** of the **DNA sample** extracted from the Negative Control to the tube labeled C– (Negative Control of Extraction).

C+ – Add **10 µl** of **DNA calibrator PIC2 *HBV*** to the tube labeled C+ (Positive Control of Amplification).

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

NCA – Add **10 µl** of **Buffer for elution** to the tube labeled NCA (Negative Control of Amplification).

8.2.2. Amplification

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

Table 4

AmpliSens-2 RG program for rotor-type instruments[4]

Step	Temperature, °C	Time	Fluorescence detection	Cycles
Hold 1	**50**	**15 min**	**–**	**1**
Hold 2	**95**	**15 min**	**–**	**1**
Cycling 1	**95**	**5 s**	**–**	**5**
	60	**20 s**	**–**	
	72	**15 s**	**–**	
Cycling 2	**95**	**5 s**	**–**	**40**
	60	**20 s**	**FAM/Green, JOE/Yellow**	
	72	**15 s**	**–**	

NOTE: Any combination of the tests can be performed in one instrument simultaneously with the use of the unified amplification program (for example, with the tests for *HDV*, *HCV*-genotyping). Step 1 (50 °C - 15 min) can be omitted in the case of simultaneous carrying out tests for detection of *HBV* DNA.

Table 5

AmpliSens-2 iQ program for plate-type instruments[5]

Step	Temperature, °C	Time	Fluorescence 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 can be performed in one instrument simultaneously with the use of the unified amplification program (for example, with the tests for *HDV*, *HCV*-genotyping). Step 1 (50 °C – 15 min) can be omitted in the case of simultaneous carrying out tests for detection of *HBV* DNA.

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin*.
3. 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.
Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument.

4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

[1] Number of test samples + 2 controls of DNA extraction + 1 control of PCR (N+3, N - number of test samples).
[2] 12-tube panel for extraction.
[3] 24-tube panel for extraction.
[4] For example, Rotor-Gene 3000/6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN GmbH, Germany).
[5] For example, CFX96 (Bio-Rad Laboratories, Inc., USA).

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:

Table 6

Channel for the fluorophore	FAM	JOE
Amplification product	Internal Control STI-87 (IC) cDNA	*HBV* DNA

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 DNA sample in the corresponding column of the results grid.
Principle of interpretation is the following:

Table 7

Results interpretation for the test samples

***Ct* value in the channel for the fluorophore**		**Result**
FAM	**JOE**	
< boundary value	absent or > boundary value	***HBV* DNA is NOT detected**
> or < boundary value	< boundary value	***HBV* DNA is detected**
absent or > boundary value	absent or > boundary value	**Invalid***

* In case of **invalid** result, the PCR analysis should be repeated starting from the RNA extraction stage for the corresponding test sample.

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

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 8).

Table 8

Results for controls

Control	**Stage for control**	***Ct* value in the channel for fluorophore**	
		FAM	**JOE**
C–	DNA extraction	≤boundary value	absent
PCE	DNA extraction	≤boundary value	≤boundary value
NCA	Amplification	absent	absent
C+	Amplification	≤boundary value	≤boundary value

Interpretation of some test samples is not possible if the results for the controls deviate from the results specified above (see *10.Troubleshooting*).

10. TROUBLESHOOTING

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

1. For the Positive Control of Extraction (PCE):
   a. The *Ct* value is absent or exceeds the boundary value in the channel for the JOE fluorophore. It is impossible to interpret the results for samples in which *HBV* DNA is not detected. It is necessary to repeat the PCR analysis for all samples in which *HBV* DNA is not detected, starting from the DNA extraction stage.
   b. The *Ct* value is absent or exceeds the boundary value in the channel for the FAM fluorophore. It is impossible to interpret the results for the test samples. It is necessary to repeat the PCR analysis for all samples starting from the DNA extraction stage.
2. For the Positive Control of PCR (C+):
   a. The *Ct* value is absent or exceeds the boundary value in the channel for the JOE fluorophore. It is impossible to interpret the results for samples in which *HBV* DNA is not detected. It is necessary to repeat the PCR for all samples in which *HBV* DNA is not detected.
   b. The *Ct* value is absent or exceeds the boundary value in the channel for the FAM fluorophore. The interpretation of the results for the test samples should be carried out according to the table 7.
3. For the Negative Control of Extraction (C–):
   a. The *Ct* value is determined in the channel for the JOE fluorophore. The contamination of laboratory with amplification fragments or contamination of reagents / test samples is probable at any stage of PCR analysis. It is impossible to interpret the results for samples in which *HBV* DNA is detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis should be repeated for all samples in which *HBV* DNA is detected starting from the DNA extraction stage.
   b. The *Ct* value in the channel for the FAM fluorophore is absent or exceeds the boundary value. It is not possible to interpret the results for the tested samples. The PCR analysis should be repeated for all samples starting from the DNA extraction stage.
4. For the Negative Control of PCR (NCA):
   a. The *Ct* value is determined in the channel for the JOE fluorophore. The contamination of laboratory with amplification fragments or contamination of reagents / test samples is probable at any stage of PCR analysis. It is impossible to interpret the results for samples in which *HBV* DNA is detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis should be repeated for all samples in which *HBV* DNA is detected.
   b. The *Ct* value in the channel for the FAM fluorophore is absent or exceeds the boundary value. The contamination of laboratory with amplification fragments or contamination of reagents / test samples is probable at any stage of PCR analysis. It is impossible to interpret the results for samples in which *HBV* DNA is detected. Measures for detecting and elimination of contamination source must be taken. The interpretation of the results for the test samples should be carried out according to the table 7.
5. If 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.

11. TRANSPORTATION

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

NOTE: DNA calibrator PIC2 *HBV*, Positive Control-1-*HBV* and Internal Control STI-87 (IC) should not be frozen/thawed more than twice. After thawing, these controls should be stored at 2–8ºC for at most 6 months.

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

13. SPECIFICATIONS

13.1. Analytical sensitivity (detection limit)

Table 9

Test material	Volume of sample for extraction, µl	DNA extraction kit	Analytical sensitivity (detection limit), IU/ml
Blood plasma	100	RIBO-sorb	100
		RIBO-prep	50
	200	MAGNO-sorb	50
	1000	MAGNO-sorb	10

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

13.2. Analytical specificity

The analytical specificity of **AmpliSens® *HBV*-FRT** PCR kit is ensured by selection of specific primers and probes and strict reaction conditions. The primers and probes were tested for possible homologies to all sequences deposited in gene banks by sequence comparison analysis.
The reagent kit detects *HBV* DNA.
Analytical specificity of the reagent kit was proved by adding human DNA to the reaction as well as genomic DNA/RNA of the following microorganisms and viruses at a concentration of at least $1x10^3$ copies/ml or higher: hepatitis A virus (*HAV*); hepatitis C virus (*HCV*); hepatitis D virus (*HDV*); human immunodeficiency virus type1 (*HIV*1); cytomegalovirus (*CMV*); Epstein-Barr virus (EBV); herpes simplex virus types 1 and 2 (*HSV*-1,2); *varicella-zoster* virus (*VZV*); human herpes virus type 6 (*HHV*-6); *Parvovirus* B19; adenovirus type 2 (*HadV*-2); *Escherichia coli*; *Staphylococcus aureus; Streptococcus pyogenes, Streptococcus agalactiae*.
There were no nonspecific responses in tests with RNA/DNA of the above-mentioned microorganisms/viruses, as well as human DNA.
The information about interfering substances is specified in the *Interfering substances and limitations of using test material samples*.

13.3. Reproducibility and repeatability

Repeatability and reproducibility were determined by testing positive and negative model samples. Positive samples were dilutions of quality control sample (QCS) containing *HBV* DNA with a concentration above detection limit. Negative control (C–) reagent was used as a negative sample.
Repeatability conditions included testing in the same laboratory, by the same operator, using the same equipment within a short period of time. Reproducibility conditions included testing in two independent laboratories, by different operators, on different days, on different equipment and different reagent kit series. The results are presented in Table 10.

Table 10

Sample type	Repeatability		Reproducibility	
	Number of samples	Agreement of results, %	Number of samples	Agreement of results, %
Positive	10	100	40	100
Negative	10	100	40	100

14. REFERENCES

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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*-FRT** 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
31.05.10	7.1. DNA Isolation	Conditions of storage of purified DNA are changed
	Page footer	Reference number is changed from R-V5-Mod(RG,iQ,Mx,Dt)-E to R-V5-Mod(RG,iQ,Mx,Dt)-CE
	3. Content, text	Name of Positive Control of amplification is changed from KB2 to PIC2
03.08.10	3. Content	The number of Positive Control PIC2 *HBV* (C+) tubes is changed from 1 to 4
10.12.10	Through the text	Name of Positive Control of amplification is changed from Positive Control PIC2 *HBV* (C+) to DNA calibrator PIC2 *HBV*
03.07.11 RT	Cover page	The phrase "For Professional Use Only" was added
	Content	New sections "Working Conditions" and "Transportation" were added
		The "Explanation of Symbols" section was renamed to "Key to Symbols Used"

VER	Location of changes	Essence of changes
	Stability and Storage	The information about the shelf life of open reagents was added
		Information that PCR-mix-1-FL is to be stored away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
	13. Specifications 13.1. Sensitivity	The table of analytical sensitivity was corrected in accordance with Russian instruction
15.09.11 RT	8. Protocol 8.1. DNA extraction	The information about using RIBO-prep kit was added
26.09.11 LA	8.2.2. Amplification	Notes below amplification program tables were corrected
13.06.12 LA	Cover page	Symbol IVD was replaced by RUO symbol
	16. Key to symbols used	
19.06.12 LA	8.1. DNA Isolation	Reference number of MAGNO-sorb reagent kit was changed from K2-16-1000 to K2-16-1000-CE
		Information about extraction with MAGNO-sorb is added
04.02.14 ME	8.1. DNA extraction	The chapter DNA isolation was renamed to DNA extraction. The information about using EM-plus reagent kit was deleted. The chapter was rewritten
	8.2. Preparing the PCR	Table 2 was added from Appendix. The tables through the text was numerated
	10. Data analysis	The chapter was rewritten
	11. Troubleshooting	The chapter was corrected in accordance with Russian instruction
	14. References	The reference for Guidelines was corrected
	8.1. DNA extraction	Information about preparing the controls of extraction was added
	13.2. Specificity	The phrase “The clinical specificity of AmpliSens® *HBV*-FRT PCR kit was confirmed in laboratory clinical trials” was deleted
03.04.15 ME	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”. Clinical material was changed to biological
02.08.17 ME	8.1. DNA extraction	Information about preparing the controls of extraction was added
	13.2. Specificity	The phrase “The clinical specificity of AmpliSens® *HBV*-FRT PCR kit was confirmed in laboratory clinical trials” was deleted
06.09.18 EM	3. Content	The colour of the reagent was specified
16.07.20 KK	Through the text	The text formatting was changed
	Footer	The phrase “For research use only. Not for diagnostic procedures” was added
14.08.20 KK	2. Principle of PCR detection	The table with targets was added
02.11.20 KK	2. Principle of PCR detection	Edits by template in section 2. Principle of PCR detection
11.02.21 KK	Footer	REF R-V5-Mod(RG,iQ,Mx,Dt)-CE-B was deleted
	3. Content	The form in bulk was deleted
11.06.24 PM	Through the text	Corrections according to the template
		Name of PCR-mix-2-FRT is changed to PCR-buffer-B
	4. Additional requirements	The section was actualized and updated with materials and instruments. Rotor-Gene Q and CFX 96 were added. Mx3000P and iCycler iQ5 were deleted
	6. Sampling and handling	The information about sampling and handling was expanded. The subsection *Interfering substances and limitations of using test material samples* was added
	8. Protocol	NucliSENS easyMAG automated was deleted The information about extraction using RIBO-sorb, RIBO-prep and MAGNO-sorb extraction kits was updated.
	9. Data analysis	Information on the correspondence of the amplification product and channels for the fluorophore, the principle of results interpretation for the test samples and controls are presented in tables
	10. Troubleshooting	The section was rewritten
	13. Specifications	The “13.1. Analytical sensitivity (detection limit)” and “13.2. Analytical specificity” subsections were actualized. The “13.3. Reproducibility and repeatability” subsection was added
	14. References	The section was actualized

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