RUO

For Professional Use Only

AmpliSens® *HBV*-FRT

PCR kit

Instruction Manual

AmpliSens®

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Science “Central Research
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1. INTENDED USE

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

For research use only. Not for diagnostic procedures.

2. PRINCIPLE OF PCR DETECTION

Hepatitis B virus (*HBV*) DNA is isolated from blood plasma together with the internal control sample (IC). The latter must be used in the isolation procedure to control the isolation of each individual sample and to detect possible reaction inhibition. *HBV* DNA detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *HBV* 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 monitoring of fluorescence intensities during the real-time PCR allows detection of the amplified 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 IC amplification product is detected in the channel for the FAM fluorophore. The *HBV* DNA amplification product is detected in the channel for the JOE fluorophore. The Positive Control of Extraction, **Positive Control-1-*HBV***, is detected in the channels for the FAM (IC) and JOE (*HBV*) fluorophores. The Positive Control of Amplification, **DNA calibrator PIC2 *HBV***, is a complex control for *HBV* and IC. It is detected in the channels for the FAM (IC) and JOE (*HBV*) fluorophores.

3. CONTENT

AmpliSens® *HBV*-FRT PCR kit is produced in 2 forms:

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

AmpliSens® *HBV*-FRT PCR kit variant FRT in bulk[1], **REF** R-V5-Mod(RG,iQ,Mx,Dt)-CE-B.

[1] In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

AmpliSens® *HBV*-FRT PCR kit includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *HBV*	clear liquid from colorless to light lilac colour	0.3	4 tubes
PCR-mix-2-FRT	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.

AmpliSens® *HBV*-FRT PCR kit is intended for 112 amplification reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit or DNA extraction automatic station.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase/DNase-free pipette tips with aerosol barriers (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); 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 at 2 to 8 °C.
- Deep-freezer at minus 24 to minus 16 °C.
- Reservoir for used tips.

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 (samples, 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 afterward.
- 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 accordance 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 (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment, and reagents to the area where the previous step was performed.

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 detail in the manufacture's handbook [1]. It is recommended that this handbook is read before starting the work.

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

- *Peripheral blood plasma*.

Blood samples are taken after overnight fasting into tubes with 3% EDTA solution (1 : 20). Closed tubes with blood are turned several times upside down and back again. Blood plasma should be taken and transferred to new tubes within 6 h after taking blood. For this purpose, tubes with blood are centrifuged at 800–1600 g for 20 min. Blood plasma can be stored unfrozen (at 2–8 °C) for at most 3 days or frozen (at or below minus 68 °C) for a long time.

In some cases, blood serum can be used. In this case, the analytical sensitivity of the reagent kit is retained; however, the clinical sensitivity may be significantly decreased as a result of precipitation of viral particles during blood clot retraction. Blood serum can be stored unfrozen (at 2–8 °C) for at most 3 days or frozen (at ≤ minus 68 °C) for a long time.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA extraction

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

- **RIBO-sorb**, REF K2-1-Et-100-CE
- **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.

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

- Add **10 µl** of **Internal Control STI-87** to each tube and then add **450 µl** of **Lysis Solution**.
- It is allowed to mix the **Lysis Solution** and **Internal Control STI-87 (IC)** in a separate sterile vial (450 µl of **Lysis Solution** and 10 µl of **Internal Control STI-87** per sample) and then transfer 450 µl of mixture to each prepared 1.5-ml tube to simplify the extraction procedure in case of great quantity of samples.
- When extracting sample to carry out several analyses (simultaneous extraction of nucleic acids for detection of *HDV* RNA, *HCV* RNA, *HGV* RNA, *HBV* DNA, and *HIV* RNA as well as *HCV*-genotyping can be done), add all required IC preparations (as its shown in RIBO-sorb instruction manual).
- For each panel 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–).**
- after addition of biological and control samples to **Lysis Solution** warm the mixture at 60 °C for 10 min prior to sorbent addition.

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

- It is allowed to mix the **Solution for Lysis** and **Internal Control STI-87 (IC)** in a separate sterile vial (300 µl of **Solution for Lysis** and 10 µl of **Internal Control STI-87** per sample) and then transfer 300 µl of mixture to each prepared 1.5-ml tube to simplify the extraction procedure in case of great quantity of samples.
- When extracting sample to carry out several analyses (simultaneous extraction of nucleic acids for detection of *HDV* RNA, *HCV* RNA, *HGV* RNA, *HBV* DNA, and *HIV* RNA as well as *HCV*-genotyping can be done), add all required IC preparations (as its shown in RIBO-prep instruction manual).
- For each panel 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–).**

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

- In case of DNA extraction from blood plasma sample of 1000 µl, the volume of the **Internal Control STI-87 (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.
- When extracting sample to carry out several analyses (simultaneous extraction of nucleic acids for detection of *HDV* RNA, *HCV* RNA, *HGV* RNA, *HBV* DNA, and *HIV* RNA as well as *HCV*-genotyping can be done), add all required IC preparations (as its shown in MAGNO-sorb instruction manual).
- For each panel 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–).**
- The volume of **Buffer for elution** required for extraction from both 1000 and 200 µl of blood plasma samples is **70 µl**.

<u>If using NucliSENS easyMAG automated system:</u>

- Use protocols and reagents allowed carrying out RNA/DNA extraction from blood plasma and serum in volume from 0.1 to 1 ml.
- **Internal Control STI-87 (IC)** (10 µl per sample) addition to the samples or lysis solution before beginning of the extraction is required.
- When extracting sample to carry out several analyses (simultaneous extraction of nucleic acids for detection of *HDV* RNA, *HCV* RNA, *HGV* RNA, *HBV* DNA, and *HIV* RNA as well as *HCV*-genotyping can be done), add all required IC preparations (by analogy).
- For each panel 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–).**
- Set the eluate volume as 50-60 µl (up to 100 µl).
- Both *On-board* and *Off-board* Lysis Buffer Dispensing and Lysis Incubation modes can be used.
  For details, see the Guidelines [2].

The purified DNA can be stored at 2–8 °C for one week and at temperatures not higher than minus 16 °C for one year.

8.2. Preparing the PCR

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

All components of the reaction mixture should be mixed immediately before use. Mix reagents for the required number of reactions for experimental and control samples according to table 1.

8.2.1 Preparing tubes for PCR

1. Before starting work, thaw and thoroughly vortex all reagents of the kit. Make sure that there are no drops on the caps of the tubes.
2. Take the required number of 0.2-ml amplification tubes for biological and control samples (two controls of extraction and one control of amplification. The type of tubes depends on the real-time PCR instrument used for analysis.
3. **To prepare the reaction mixture**, mix the reagents (**10 µl of PCR-mix-1-FL *HBV,* 5 µl of PCR-mix-2-FRT,** and **0.5 µl of Polymerase (TaqF)** per one reaction) in a new sterile tube (see also table 1). Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

Table 1

Scheme of reaction mixture preparation

		Reaction volume (with allowance for one extra sample)		
Reagent volume for one reaction, µl		**10.00**	**5.00**	**0.50**
Number of biological samples	Number of PCR reactions[2]	PCR-mix-1-FL *HBV*	PCR-mix-2-FRT	Polymerase (TaqF)
4	**7**	**80**	**40**	**4.0**
6[3]	**9**	**100**	**50**	**5.0**
8	**11**	**120**	**60**	**6.0**
10[4]	**13**	**140**	**70**	**7.0**
12	**15**	**160**	**80**	**8.0**
14[5]	**17**	**180**	**90**	**9.0**
16	**19**	**200**	**100**	**10.0**
18	**21**	**220**	**110**	**11.0**
20	**23**	**240**	**120**	**12.0**
22[6]	**25**	**260**	**130**	**13.0**
34	**37**	**380**	**190**	**19.0**
46	**49**	**500**	**250**	**25.0**

4. Add **15 µl** of the prepared reaction mixture to each PCR tube.

5. Add **10 µl** of **DNA samples** extracted from the biological samples to each PCR tube.

Avoid transferring sorbent together with the DNA sample in case of extraction using RIBO-sorb and MAGNO-sorb kits or the NucliSENS easyMAG automated system.

6. Run 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).

Make sure that there are no drops on the tube walls, otherwise vortex tubes briefly.

[2] Number of biological samples + 2 controls of extraction + 1 control of PCR (N+3, N - number of biological samples)
[3] Extraction in one stripe in NucliSENS easyMAG (8 tubes)
[4] 12-tube panel for extraction
[5] Extraction in two stripes in NucliSENS easyMAG (16 tubes)
[6] 24-tube panel for extraction, extraction in three stripes in NucliSENS easyMAG

8.2.2 Amplification

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

Table 2

AmpliSens-2 RG program for rotor-type instruments[7]

Step	***Temperature*, °C**	***Time***	***Fluorescence detection***	**Cycles**
1 (Hold)	50	15 min	–	1
2 (Hold)	95	15 min	–	1
3 (Cycling 1)	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
4 (Cycling 2)	95	5 s	–	40
	60	20 s	FAM, JOE, ROX, Cy5	
	72	15 s	–	

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.

Channels ROX and Cy5 are switched on when necessary (only in MULTIPRIME assays).

Table 3

AmpliSens-2 iQ program for plate-type instruments[8]

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, ROX, Cy5	
	72	15 s	–	

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.

[7] For example, Rotor-Gene 3000 or 6000 (Corbett Research, Australia).
[8] For example, iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), DT-96 (DNA-Technology, Russia), or equivalent.

Channels ROX and Cy5 are switched on when necessary (only in MULTIPRIME assays)

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.

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:

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

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:

- The sample is considered **positive** for *HBV* DNA if the *Ct* value detected in the channel for the JOE fluorophore does not exceed the boundary value specified in the *Important Product Information Bulletin.*
- The sample is considered **negative** for *HBV* DNA if the *Ct* value in the channel for the JOE fluorophore is absent or if the *Ct* value detected in the channel for the JOE fluorophore is greater than the specified boundary value and the *Ct* value in the channel for the FAM fluorophore does not exceed the boundary value specified in the *Important Product Information Bulletin*.
- The sample is considered **equivocal** if an equivocal result is obtained in any of the channels. In this case, PCR analysis of this sample should be repeated once again.

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

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	*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

10. TROUBLESHOOTING

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

- If the Ct value for PCE or C+ in the channel for the JOE fluorophore exceeds the specified boundary value, analysis of all samples in which *HBV* DNA was not detected should be repeated once again starting from the DNA extraction stage.
- If the Ct value for NCA and/or C– in the channel for the JOE fluorophore is detected, analysis of all samples in which *HBV* DNA was detected should be repeated once again starting from the DNA extraction stage.

11. TRANSPORTATION

AmpliSens® *HBV*-FRT PCR kit should be transported at 2–8 °C 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.

DNA calibrator PIC2 *HBV*, Positive Control-*1-HBV* and the 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.

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

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *HBV*-FRT** PCR kit is specified in the table below.

Volume of sample for extraction, µl	DNA extraction kit	Analytical sensitivity, IU/ml
100	RIBO-sorb	100
	RIBO-prep NucliSENS easyMAG	50
200	MAGNO-sorb	50
1000	MAGNO-sorb	10
	NucliSENS easyMAG	5

The claimed analytical features of **AmpliSens® *HBV*-FRT** PCR kit are guaranteed only when additional reagents kits **MAGNO-sorb, RIBO-sorb**, or **RIBO-prep** (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology") are used.

13.2. 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 as well as with genomic DNA/RNA of the following organisms and viruses: hepatitis A virus; hepatitis C virus; hepatitis D 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*. Cross-reactions for the above-mentioned organisms and viruses have not been detected.

14. REFERENCES

1. Handbook "Sampling, Transportation, 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*-FRT**, **AmpliSens® *HDV*-FRT**, **AmpliSens® *HBV*-FRT**, **AmpliSens® *HGV*-FRT** PCR kits 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*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Sufficient for
LOT	Batch code		Expiration Date
RUO	Research use only		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**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

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”
	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 1 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

VER	Location of changes	Essence of changes
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
	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
02.08.17 ME	Content	The form in bulk was added
	Footer	**REF** R-V5-Mod(RG,iQ,Mx,Dt)-CE-B was added
06.09.18 EM	3. Content	The colour of the reagent was specified