AmpliSens® *HBV / HDV*-FRT PCR kit

RUO

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	⚠	Caution
LOT	Batch code	Σ	Contains sufficient for <n> tests
RUO	Research use only	⏳	Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C–	Negative control of extraction
PCE	Positive Control of Extraction	C+$_{HBV / HDV-FL}$	Positive Control of Amplification
		IC	Internal control

1. INTENDED USE

AmpliSens® *HBV / HDV*-FRT PCR kit is not a medical device. PCR kit is an *in vitro* nucleic acid amplification test for simultaneous detection of *hepatitis B virus* (*HBV)* DNA and *hepatitis D virus* (*HDV)* RNA in biological materials (blood plasma) by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

Hepatitis B virus (*HBV)* DNA and *hepatitis D virus* (*HDV)* RNA are extracted from blood plasma together with internal control sample (IC). Detection by the polymerase chain reaction (PCR) is based on the reverse transcription of DNA/RNA and amplification of pathogen genome specific region using specific primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time PCR monitoring of the 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 / HDV*-FRT PCR kit is a qualitative test which contain the Internal Control (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.

AmpliSens® *HBV / HDV*-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically 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.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE	ROX
DNA/cDNA-target	IC DNA	*HBV* DNA	*HDV* cDNA
Target gene	Artificially synthesized sequence	gene C	untranslated region

3. CONTENT

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

variant FRT, REF R-V56(RG,iQ,Mx,Dt)-CE.

Variant FRT includes:

Reagent	Description	Volume, ml	Quantity
RT-G-mix-2	colorless clear liquid	0.015	4 tubes
RT-PCR-mix-1-FL *HBV / 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
Positive Control cDNA *HBV / HDV*-FL (C+$_{HBV / HDV-FL}$)	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 *HBV / HDV*-rec**	colorless clear liquid	0.06	4 tubes
Internal Control *ICZ*-rec (IC)***	colorless clear liquid	0.28	4 tubes

* 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 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- RNA/DNA extraction kit or RNA/DNA extraction automatic station.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase/DNase-free pipette tips with filters (up to 200 µl).
- Tube racks.
- Centrifuge/vortex mixer.
- 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 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 (SDS) 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

NOTE 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® *HBV / HDV*-FRT PCR kit is intended for the reverse transcription of RNA and amplification of cDNA extracted by RNA/DNA extraction kits from peripheral blood plasma.

- *Peripheral blood plasma*.

Blood samples are taken after overnight fasting into the tube with EDTA solution as anticoagulant. 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 the temperature not more than 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 for such material is the same but the clinical sensitivity can be reduced in view of viral particles coprecipitation during clot retraction. Blood serum 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.

7. WORKING CONDITIONS

AmpliSens® *HBV / HDV*-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-sorb**, REF K2-1-Et-100-CE (2 kits)
- **RIBO-prep**, REF K2-9-Et-100-CE (2 kits)
- **MAGNO-sorb**, REF K2-16-1000-CE
- Automated system NucliSENS easyMAG can also be used.

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

- Add **10 µl** of **Internal Control *ICZ*-rec** to each tube and then add **450 µl** of **Lysis Solution**.
- It is allowed to mix the **Lysis Solution** and **Internal Control *ICZ*-rec** in a separate sterile vial (450 µl of **Lysis Solution** and 10 µl of **Internal Control *ICZ*-rec** 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 the **RIBO-sorb** *Instruction manual*).
- After addition of clinical and control samples to lysis solution warm the mixture at 60 °C for 10 min prior to sorbent addition.

NOTE: **If using RIBO-prep kit**, 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 *ICZ*-rec** in a separate sterile vial (300 µl of **Solution for Lysis** and 10 µl of **Internal Control *ICZ*-rec** 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 the **RIBO-prep** *Instruction manual*).

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

- In case of RNA/DNA extraction from blood plasma sample of 1000 µl, the volume of the **Internal Control *ICZ*-rec** required for 24-tube panel is **0.28 ml**. In case of other panels and RNA/DNA extraction from blood plasma sample of 200 µl see 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 the **MAGNO-sorb** *Instruction manual*).
- To prepare the Positive Control of extraction, **PCE**, add **90 µl** of the **Negative Control (C–)** sample and **10 µl** of the **Positive Control *HBV* / *HDV*-rec** sample to a tube containing **Lysis Solution MAGNO-sorb**.
- To prepare the Negative Control of extraction, **C–**, add **100 µl** of the **Negative Control (C–)** sample to a 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**.

NOTE: If NucliSENS easyMAG automated system is used:

- 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 *ICZ*-rec** (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).
- 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.
- Then the RNA extraction is completed, take the tubes from the device and carry out the RT-PCR reaction. Purified RNA can be stored at 2–8 °C for 4 hours, at not more than minus 16 °C for one month and at not more than minus 68 °C for one year.

For details, see the Guidelines [2].

8.2. Preparing PCR

Total reaction volume is **25 µl**, the volume of RNA/DNA sample is **10 µl**.

8.2.1 Preparing tubes for RT-PCR

NOTE: All components of the reaction mix should be mixed immediately before use. Mix reagents for the required number of reactions for experimental and control samples according to table 2.

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 tubes for amplification for the clinical and control samples (two controls of extraction and one control of amplification). The type of tubes depends on the PCR instrument used for analysis.
3. To prepare the reaction mixture, mix reagents **10 µl of RT-PCR-mix-1-FL *HBV* / *HDV*, 5 µl of RT-PCR-mix-2-FEP/FRT, 0.25 µl of RT-G-mix-2, 0.5 µl of polymerase (TaqF)** and **0.25 µl of TM-Revertase (MMlv)** per one reaction in a new sterile tube. Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

Table 2

Reaction mixture preparation

		Reagents volumes for specified number of samples and 1 extra reaction, µl				
Reagent volume per one reaction, µl		**10.00**	**5.00**	**0.25**	**0.50**	**0.25**
Number of clinical samples	Number of analyzed samples[1]	RT-PCR-mix-1-FL *HBV* / *HDV*	RT-PCR-mix-2-FEP/FRT	RT-G-mix-2	Polymerase (TaqF)	TM-Revertase (MMlv)
4	**7**	**80**	**40**	**2.0**	**4.0**	**2.0**
6[2]	**9**	**100**	**50**	**2.5**	**5.0**	**2.5**
8	**11**	**120**	**60**	**3.0**	**6.0**	**3.0**
10[3]	**13**	**140**	**70**	**3.5**	**7.0**	**3.5**
12	**15**	**160**	**80**	**4.0**	**8.0**	**4.0**
14[4]	**17**	**180**	**90**	**4.5**	**9.0**	**4.5**
16	**19**	**200**	**100**	**5.0**	**10.0**	**5.0**
18	**21**	**220**	**110**	**5.5**	**11.0**	**5.5**
20	**23**	**240**	**120**	**6.0**	**12.0**	**6.0**
22[5]	**25**	**260**	**130**	**6.5**	**13.0**	**6.5**
34	**37**	**380**	**190**	**9.5**	**19.0**	**9.5**
46	**49**	**500**	**250**	**12.5**	**25.0**	**12.5**

4. Transfer **15 µl** of prepared mixture into each tube.
5. Using tips with filters add **10 µl** of **RNA/DNA** obtained from clinical samples.

NOTE: When adding of RNA/DNA samples extracted by RIBO-sorb, MAGNO-sorb and NucliSENS easyMAG avoid transferring the sorbent into the reaction mix.

6. Carry out the control amplification reactions:

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

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

C+*HBV / HDV*-FL – Add **10 µl** of **Positive Control cDNA *HBV* / *HDV*-FL** to the tube labeled **C+*HBV / HDV*-FL** (Positive Control of Amplification).

To rule out possible contamination, carry out 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 3

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

Step	Temperature, °C	Time	Fluorescence detection	Cycles
1 (Hold)	50	15 min	–	1
2 (Hold)	95	15 min	–	1
3 (Cycling)	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	–	

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).

NOTE Channel Cy5 is switched on when necessary (only in MULTIPRIME assays).

Table 4

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

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	–	

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).

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.

[1] Number of clinical samples + 2 controls of extraction + 1 control of RT-PCR, (N+3, N – number of clinical samples).
[2] Extraction of one strip by NucliSENS easyMAG device (8 tubes).
[3] 12-tube panel for extraction.
[4] Extraction of two strips in NucliSENS easyMAG device (16 tubes).
[5] 24-tube panel for extraction, extraction of three strips in NucliSENS easyMAG device.
[6] For example, Rotor-Gene 3000 or 6000 (Corbett Research, Australia).
[7] For example, iCycler iQ5 (BioRad, USA), Mx3000P (Stratagene, 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:

- 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.
- The signal of the *HDV* cDNA amplification product is detected in the channel for the ROX 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 sample in the corresponding column of the results grid.

Principle of interpretation is the following:

- The sample is considered **positive** for *HBV* 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 **positive** for *HDV* RNA if the *Ct* value detected in the channel for the ROX fluorophore does not exceed the boundary value specified in the *Important Product Information Bulletin*.
- The sample is considered **negative** for *HDV* RNA if the *Ct* value in the channel for the ROX fluorophore is absent or if the *Ct* value detected in the channel for the ROX 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 to be equivocal in case of equivocal result in any channel. The PCR analysis is recommended to be repeated.

NOTE: 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 5).

Table 5

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore		
		FAM	JOE	ROX
C–	RNA/DNA extraction, Amplification	≤boundary value	Absent	Absent
PCE	RNA/DNA extraction, Amplification	≤boundary value	≤boundary value	≤boundary value
C+$_{HBV\,/\,HDV\text{-}FL}$	Amplification	≤boundary value	≤boundary value	≤boundary value
NCA	Amplification	Absent	Absent	Absent

10. TROUBLESHOOTING

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

1. If for Positive Controls (C+$_{HBV\,/\,HDV\text{-}FL}$ and PCE) the *Ct* value is absent or exceeds the boundary value in the channel for the JOE fluorophore, the analysis of all samples in which *HBV* DNA was not detected should be repeated once again starting from the extraction stage.
2. If for Positive Controls (C+$_{HBV\,/\,HDV\text{-}FL}$ and PCE) the *Ct* value is absent or exceeds the boundary value in the channel for the ROX fluorophore, the analysis of all samples in which *HDV* RNA was not detected should be repeated once again starting from the extraction stage.
3. If the *Ct* value is determined for negative Controls (C– and NCA) in the channels for the JOE and ROX fluorophores, the PCR of samples in which *HBV* DNA or *HDV* RNA was detected should be repeated starting from the extraction stage.

11. TRANSPORTATION

AmpliSens® *HBV* / *HDV*-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* / *HDV*-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* / *HDV*-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: Positive Control cDNA *HBV* / *HDV*-FL, Positive Control *HBV* / *HDV*-rec, and Internal Control *ICZ*-rec should not be frozen/thawed more than twice.
After thawing, Positive Control cDNA *HBV* / *HDV*-FL,
Positive Control *HBV* / *HDV*-rec, and Internal Control *ICZ*-rec are to be stored at 2-8 °C for up to 6 months.

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

13. SPECIFICATIONS

13.1. Analytical sensitivity

Analytical sensitivity of **AmpliSens® *HBV* / *HDV*-FRT** PCR kit is given the table below.

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

13.2. Analytical specificity

The analytical specificity of **AmpliSens® *HBV* / *HDV*-FRT** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked 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 B 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; adenovirus types 2, 3, and 7; *Escherichia coli*; *Staphylococcus aureus; Streptococcus pyogenes, S.agalactiae;* and *Homo sapiens*. Cross reactions for marked organisms and viruses are not registered.

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.
2. Guidelines to **AmpliSens® *HBV* / *HDV*-FRT** PCR kit for simultaneous detection of *hepatitis B virus* (*HBV)* DNA and *hepatitis D virus* (*HDV)* RNA in the biological materials 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* / *HDV*-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
29.11.10	Footer	Catalogue number R-V56-(RG,iQ,Mx,Dt) is changed into R-V56(RG,iQ,Mx,Dt)-CE
30.11.10	Sampling and handling	Sentence «Blood samples are taken after overnight fasting into tubes with 3% EDTA solution (1 : 20)» is changed into «Blood samples are taken after overnight fasting into the tube with EDTA solution as anticoagulant»
	Through the text	MAGNO-sorb mention was deleted
		Corrections through the text
		Abreviation C+$_{HBV\,/\,HDV\text{-}FL}$ is added for Positive Control cDNA *HBV* / *HDV*-FL
21.03.11 RT	Stability and storage	The phrase about keeping away from light of RT-PCR-mix-1-FL *HBV* / *HDV* was added
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
	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”
15.09.11 RT	8. PROTOCOL 8.1. RNA isolation	The information about using RIBO-prep kit was added
21.06.12 BO	Cover Page	IVD-symbol was changed to RUO
	Text	“tips with aerosol barriers” was changed to “tips with filters”
		Isolation was changed to extraction
	Sensitivity	Sensitivity for sample 200 µl was added
	8.1. RNA Extraction	Information about MAGNO-sorb was added
10.07.13 FN	Content	The second form was added – PCR kit variant FRT in bulk
	Footer	REF R-V56(RG,iQ,Mx,Dt)-CE-B was added
04.03.14 ME	8.1. 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 rewritten
	14. References	The references was corrected
14.05.15 PM	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”
	13.2. Specificity	The phrase “The clinical specificity of AmpliSens® *HBV* / *HDV*-FRT PCR kit was confirmed in laboratory clinical trials” was deleted
16.07.20 MM	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	Fixes by template
16.11.20 MM	Content	The second form was deleted – PCR kit variant FRT in bulk
	Footer	REF R-V56(RG,iQ,Mx,Dt)-CE-B was deleted
20.10.21 MM	8.1. RNA extraction	The RIBO-sorb REF K2-1-Et-50-CE was changed to RIBO-sorb REF K2-1-Et-100-CE; the RIBO-prep REF K2-9-Et-50-CE was changed to RIBO-prep REF K2-9-Et-100-CE

AmpliSens®

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