AmpliSens® *HGV*-FRT PCR kit

CE IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Use-by Date
IVD	*In vitro* diagnostic medical device		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+$_{HGV\text{-}FL}$**	Positive control of amplification
	Caution	**PCE**	Positive control of extraction
EC REP	Authorized representative in the European Community	**IC**	Internal control

1. INTENDED USE

AmpliSens® *HGV*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *hepatitis G virus* (*HGV)* RNA in clinical material (blood plasma) using real-time hybridization-fluorescence detection.

NOTE: The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

HGV detection by the polymerase chain reaction (PCR) is based on the *HGV* RNA extraction from blood plasma together with the internal control sample (IC); the reverse transcription of *HGV* RNA and the amplification of the pathogen genome specific region using special *HGV* primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that 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® *HGV*-FRT PCR kit is a qualitative test that contains 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® *HGV*-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The IC amplification product is detected in the channel for the FAM fluorophore. The *HGV* cDNA amplification product is detected in the channel for the JOE fluorophore. The Positive Control of Extraction, **Positive Control *HGV*-FL-rec**, is detected in the channels for the FAM (IC) and JOE (*HGV*) fluorophores. The Positive Control of Amplification, **Positive Control cDNA *HGV*-FL (C+$_{HGV\text{-}FL}$)**, is a complex control for *HGV* and IC. It is detected in the channels for the FAM (IC) and JOE (*HGV*) fluorophores.

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

Table 1

Channel for fluorophore	FAM	JOE
cDNA-target	Internal Control-FL (IC) cDNA	*HGV* cDNA
Target gene	Artificially synthesized sequence	5'UTR

3. CONTENT

AmpliSens® *HGV*-FRT PCR kit is produced in 1 form:
variant FRT-50 F, REF R-V2-50-F(RG,iQ,Mx,Dt)-CE.

Variant FRT-50 F includes:

Reagent	Description	Volume, ml	Quantity
RT-G-mix-2	colorless clear liquid	0.015	1 tube
RT-PCR-mix-1-FL *HGV*	clear liquid from colorless to light lilac colour	0.6	1 tube
RT-PCR-mix-2-FEP/FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
TM-Revertase (MMlv)	colorless clear liquid	0.015	1 tube
Positive Control cDNA *HGV*-FL (C+$_{HGV\text{-}FL}$)	colorless clear liquid	0.1	1 tube
Buffer for elution	colorless clear liquid	1.2	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Positive Control *HGV*-FL-rec**	colorless clear liquid	0.1	1 tube
Internal Control *ICZ*-rec (IC)***	colorless clear liquid	0.28	2 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.
*** add **10 µl** of **Internal Control ICZ-rec (IC)** during the RNA extraction procedure directly to the sample/lysis solution before the extraction.

Variant FRT-50 F is intended for 55 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 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):
- 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
- 0.2-ml PCR tubes with flat caps PCR tubes if a rotor-type instrument is used.
- Refrigerator at the temperature from 2 to 8 °C.
- Deep-freezer at the temperature from 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 (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid 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.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

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

Not for use in the Russian Federation

6. SAMPLING AND HANDLING

NOTE: Obtaining samples of biological materials 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® *HGV*-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 (serum).

- *Peripheral blood plasma (serum)*.

Blood samples are taken into the tube with 3% EDTA solution (20 parts of blood to 1 part of EDTA). 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. After that blood plasma should be taken and transferred to new disposable tubes.
To obtain serum, tubes with blood should be incubated at room temperature to allow complete clot formation. Then tubes are centrifuged at 800–1600 g for 10 min. After that serum should be taken and transferred to new disposable tubes.
Blood plasma (serum) 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.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. RNA extraction

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

- **RIBO-prep**, REF K2-9-Et-50-CE;
- NucliSENS easyMAG automated nucleic acid extraction system (bioMérieux, France) can also be used.

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 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 *HGV*-FL-rec**. To the tube labelled C– add **100 µl** of **Negative Control (C–).**

NOTE: **If using NucliSENS easyMAG automated system**:

- 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).
- 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 *HGV*-FL-rec**. To the tube labelled C– add **100 µl** of **Negative Control (C–).**
- Set the elution 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 the temperature 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].

See Guidelines [2] for details.
The purified RNA can be stored at 2–8 °C for 4 hours, at the temperature not more than minus 16 °C for one month or at the temperature not more than minus 68 °C for one year.

8.2. Preparing the reverse transcription PCR

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

8.2.1 Preparing tubes for 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 Thaw 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 two controls of amplification). The type of tubes depends on the PCR instrument used for analysis.
3 To prepare the reaction mixture, mix reagents per one reaction in a new sterile tube: **10 µl of RT-PCR-mix-1-FL *HGV*, 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)**. Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

Table 2

Reaction mixture preparation

		Reagent volumes, 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 *HGV*	RT-PCR-mix-2-FEP/FRT	RT-G-mix-2	Polymerase (TaqF)	TM-Revertase (MMlv)
4	**8**	**80**	**40**	**2.0**	**4.0**	**2.0**
6[2]	**10**	**100**	**50**	**2.5**	**5.0**	**2.5**
8	**12**	**120**	**60**	**3.0**	**6.0**	**3.0**
10[3]	**14**	**140**	**70**	**3.5**	**7.0**	**3.5**
12	**16**	**160**	**80**	**4.0**	**8.0**	**4.0**
14[4]	**18**	**180**	**90**	**4.5**	**9.0**	**4.5**
16	**20**	**200**	**100**	**5.0**	**10.0**	**5.0**
18	**22**	**220**	**110**	**5.5**	**11.0**	**5.5**
20	**24**	**240**	**120**	**6.0**	**12.0**	**6.0**
22[5]	**26**	**260**	**130**	**6.5**	**13.0**	**6.5**
34	**38**	**380**	**190**	**9.5**	**19.0**	**9.5**
46	**50**	**500**	**250**	**12.5**	**25.0**	**12.5**

4 Transfer **15 µl** of the prepared mixture into each tube.
5 Add **10 µl** of **RNA** obtained from clinical samples into the prepared tubes using tips with aerosol barrier.

NOTE: When adding of RNA samples extracted by NucliSENS easyMAG it is necessary to avoid transferring of the sorbent into the reaction mix.

6 Carry out control amplification reactions:

PCE – Add **10 µl** of **RNA sample** extracted from the **Positive Control *HGV*-FL-rec** sample to the tube labeled PCE (Positive Control of Extraction).
C– – Add **10 µl** of **RNA sample** extracted from the **Negative Control (C–)** sample to the tube labeled C– (Negative Control of Extraction).
NCA – Add **10 µl** of **buffer for elution** to the tube labeled NCA (Negative Control of Amplification).
C+*HGV-FL* – Add **10 µl** of **Positive Control cDNA *HGV*-FL** to the tube labeled C+*HGV-FL* (Positive Control of Amplification).

8.2.2. Amplification

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

Table 3

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

Step	Temperature, °C	Time	Fluorescence detection	Cycles
Hold	50	15 min	–	1
Hold	95	15 min	–	1
Cycling	95	5 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Cycling 2	95	5 sec	–	40
	60	20 sec	FAM, JOE, ROX, Cy5	
	72	15 sec	–	

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 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
4	95	5 sec	–	40
	60	30 sec	FAM, JOE, ROX, Cy5	
	72	15 sec	–	

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

2. Adjust the fluorescence channel sensitivity according to the Guidelines [2] and the *Important Product Information Bulletin*.
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 + 2 controls of RT-PCR, (N+4, 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 by NucliSENS easyMAG device (16 tubes)
[5] 24-tube panel for extraction, extraction of three strips by NucliSENS easyMAG device
[6] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or recommended in the Guidelines [2].
[7] For example, iCycler iQ5, Mx3000P, or those recommended in the Guidelines [2].

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 cDNA amplification product is detected in the channel for the FAM fluorophore,
– The signal of the *HGV* cDNA 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 RNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

– The sample is considered **positive** for *HGV* RNA if its *Ct* 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 *HGV* RNA 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** 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
C–	RNA extraction, Amplification	< boundary value	absent
PCE	RNA extraction, Amplification	< boundary value	< boundary value
C+$_{HGV\text{-}FL}$	Amplification	< boundary value	< boundary value
NCA	Amplification	absent	absent

10. TROUBLESHOOTING

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

1. If the *Ct* value is absent or it exceeds the specified boundary Ct value for the positive control of extraction (PCE) or the positive control of amplification (C+$_{HGV\text{-}FL}$) in the channel for the JOE fluorophore, the analysis of samples in which *HGV* RNA was not detected should be repeated starting from the RNA extraction stage.
2. If a *Ct* value is present for negative controls of extraction (C–) and/or the negative control of amplification (NCA) in the channel for the JOE fluorophore, the analysis of samples in which *HGV* RNA was detected should be repeated from the RNA extraction stage.

If you have any further questions or if encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

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

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

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *HGV*-FRT** PCR kit is given in the table below:

Table 6

Volume of sample for extraction, µl	RNA/DNA extraction kit	Analytical sensitivity, copies/ml
100	RIBO-prep NucliSENS easyMAG	500
1000	NucliSENS easyMAG	50

NOTE: The claimed analytical performance characteristics of **AmpliSens® *HGV*-FRT** PCR kit are guaranteed only when additional reagent kit **RIBO-prep** (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology") is used. NucliSENS easyMAG manufactured by bioMérieux, France can be used either.

13.2. Specificity

The analytical specificity of **AmpliSens® *HGV*-FRT** PCR kit is ensured by selection of specific primers and probes as well as by selection 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 by addition of genomic DNA/RNA of the following organisms and viruses into the reaction: H*epatitis A virus; Hepatitis B virus; Hepatitis C virus; Hepatitis D virus, Hepatitis E virus, Human immunodeficiency virus; Cytomegalovirus; Epstein-Barr virus; Herpes simplex virus* types 1 and 2; *Enterovirus* (*Coxsakie* B1, B2, B3, B4, B5, B6, *Polio* I, II, III); *Human rotavirus WA*, *Astrovirus*, *Human herpes virus* types 6 and 8; *Adenovirus* types 2, 3, and 7; and *Homo sapiens*. Cross reactions for marked organisms and viruses are not registered.

The clinical specificity of **AmpliSens® *HGV*-FRT** PCR kit was confirmed in laboratory clinical trials.

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® *HCV*-FRT, AmpliSens® *HDV*-FRT, AmpliSens® *HBV*-FRT, and 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® *HGV*-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
13.02.18 PM	3. Content	The colour of the reagent was specified
04.06.20 KK	Through the text	The text formatting was changed
	2. Principle of PCR detection	The table with targets was added
	Footer	The phrase "Not for use in the Russian Federation" was added
23.03.21 EM	—	The name, address and contact information for Authorized representative in the European Community was changed

AmpliSens®

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