



IVD For in Vitro Diagnostic Use

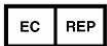
AmpliSens® HAV-EPh PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE

AmpliSens® HAV-EPh PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *hepatitis A virus* in the clinical material (blood plasma, feces) and environmental samples (concentrated water samples) by means of detection of the amplified products by agarose gel electrophoresis.

2. PRINCIPLE OF PCR ASSAY

Hepatitis A virus detection by the polymerase chain reaction (PCR) is based on the amplification of specific region of cDNA of pathogen genome using specific HAV primers. After PCR the amplified product is detected in agarose gel. AmpliSens® HAV-EPh PCR kit is qualitative test and contains the IC which must be used in the isolation procedure in order to control the isolation process of each individual specimen and to identify possible reaction inhibition.

AmpliSens® HAV-EPh PCR kit uses "hot-start", that is guaranteed by separation of nucleotides and Taq-polymerase by wax layer. Melting of wax and mix of reaction components occur only at 95°C, which greatly diminish frequency of nonspecifically primed reactions.

3. CONTENTS OF THE KIT

AmpliSens® HAV-EPh PCR kit is produced in 2 forms:

AmpliSens® HAV-EPh PCR kit variant 50 R (vials 0.5 ml), REF V4-50-R0,5-CE.

AmpliSens® HAV-EPh PCR kit variant 50 R (vials 0.2 ml), REF V4-50-R0,2-CE.

AmpliSens® HAV-EPh PCR kit variant 50 R includes:

Reagent	Description	variant 50 R	
		Volume (ml)	Amount
PCR-mix -1-R HAV ready-to-use single-dose test tubes (under wax)	colourless, clear fluid	0.005	55 vials of 0.5 or 0.2 ml
PCR-mix-2 blue	clear fluid of blue colour	0.6	1 vial
Mineral oil for PCR	colourless viscous fluid	2.0	1 vial
Positive Control cDNA HAV (C+)	colourless, clear fluid	0.1	1 vial
DNA-buffer	colourless, clear fluid	0.5	1 vial
Negative Control (C-)*	colourless, clear fluid	1.2	3 vials
Positive Control HAV-rec	colourless, clear fluid	0.03	5 vials
Internal Control HAV-rec**	colourless, clear fluid	0.06	5 vials

* must be used in the isolation procedure as Negative Control of Extraction.

** add 5 µl of Internal Control during the RNA isolation procedure directly to the sample/lysis mixture (see "RIBO-sorb", REF K2-1-50-CE or "RIBO-prep", REF K2-9-50-CE protocols).

AmpliSens® HAV-EPh PCR kit variant 50 R is sufficient for 55 reactions, including controls.

4. ADDITIONALLY REQUIRED MATERIALS, REAGENTS AND DEVICES

- Disposable powder-free gloves
- RNA isolation kit
- Detection agarose kit
- Pipettes (adjustable)
- Sterile pipette tips with aerosol filters (up to 200 µl)
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- PCR box
- Personal thermocyclers
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity
- Refrigerator for 2–8 °C with deep-freezer with temperature no less then –16°C
- Reservoir for disposed tips

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and put the new tip for every procedure.
- Store and handle amplicons separately from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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 specimens and unused reagents in accordance with local regulations.
- Specimens should be considered potentially infectious and handled in biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucose membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.



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

6. SPECIMEN COLLECTION AND HANDLING



In detail, sampling biological materials for PCR-analysis, transportation and storage is described in handbook of the manufacture [2]. It is recommended to read this handbook before beginning of the work.

AmpliSens® HAV-EPh PCR kit is intended to analyze RNA extracted with RNA isolation kits from:

- *Blood plasma (serum)*
- *Feces*
- *Concentrated water samples (wastewater, drinking, from reservoir)*

6.1. Blood plasma. Blood should be collected in a tube that contains 6% EDTA (50 µl of EDTA per 1.0 ml of blood) after overnight fasting. After the tube is filled invert it several times to ensure adequate mixing. Spin the tube at 3,000 r/min for 10 min. Remove and transfer plasma in a 1.5 ml tube using aerosol filter tip. Plasma should be collected within 6 h from the time of blood taking.

Blood serum. Blood should be collected in a tube that contains 6% EDTA (50 µl of EDTA per 1.0 ml of blood) after overnight fasting. After the tube is filled invert it several times to ensure adequate mixing. Incubate tube at room temperature until blood clot is formed. Spin at 3,000 r/min for 10 min. Remove and transfer serum in a clean tube.

6.2. Feces.

1. Pipette 0.8 ml of phosphate buffer (or sterile saline solution) in a 1.5 ml tube. Add 0.1 gram (0.1 ml) of feces into the tube using tip with aerosol filter or disposable spatula.
2. Vortex thoroughly to ensure homogenous suspension.
Omit steps 1 and 2 for liquid feces.
3. Spin the tube with prepared fecal suspension or with liquid feces at 10,000 g for 5 min. Transfer supernatant in a clean tube and use for RNA extraction.

If long-time storage of fecal suspension is necessary it should be mixed with glycerin (final concentration is 15%), incubated at room temperature for 1 h and stored frozen.

6.3. Concentrated water samples (eluates): wastewater, drinking, form reservoir

Prior to RNA extraction water samples (eluates) should be mixed on vortex. Water samples with visual impurities or colour should be centrifuged (at 10,000 r/min for 1 min) after vortex. Use supernatant for RNA extraction.



Only one freeze-thaw cycle of clinical material is allowed.

7. PROTOCOL

7.1. RNA Isolation

Different manufacturers offer RNA isolation kits. We recommend following nucleic acid extraction kits:

- "RIBO-sorb", [REF](#) K2-1-50-CE.

[REF](#) V4-50-R0,5-CE or V4-50-R0,2-CE

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- "RIBO-prep", [REF](#) K2-9-50-CE.



Please carry out the RNA isolation according to the manufacturer instruction.



When extracting RNA from feces take 50 µl of clinical sample.



When extracting RNA from fecal or water samples add 50 µl of Negative Control (C-) into **each** sample/lysis solution/IC mixture.



Positive Control HAV-rec must be used during RNA isolation procedure. Add 10 µl of PC HAV-rec and 90 µl of Negative Control (C-) in the tube of Positive Control of Extraction.

7.2. Reverse transcription

Different manufacturers offer Reverse Transcription kits. We recommend following kit for complementary DNA (cDNA) synthesis from RNA:

- "REVERTA-L", [REF](#) K3-4-50-CE.



Please carry out the reverse transcription procedure according to the manufacturer instruction.

7.3. Preparing the PCR

Total reaction volume - 25 µl, volume of cDNA sample - 10 µl.

7.3.1 Preparing tubes for PCR

1. Collect the required quantity of tubes with **PCR-mix-1-R HAV** with wax for amplification of cDNA of study and control samples.
2. Add **10 µl of PCR-mix-2 blue** to the surface of wax layer, so that it wouldn't fall under the wax and mix with PCR-mix-1-R HAV.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl).

7.3.2 Amplification

Use prepared tubes for PCR. Under or immediately above the level of oil, using tips with aerosol barrier, **add 10 µl of cDNA samples**, obtained from clinical or control samples at the stage of reverse transcription.

Perform **control amplification reactions**:

NCA	Add 10 µl of DNA-buffer to the tube for Negative Control of Amplification (NCA).
C+	Add 10 µl of Positive Control cDNA HAV to the tube for Positive Control of Amplification.

Run the following program on the thermocycler (see table 1). When the temperature will reach 95°C (pause regimen), insert tubes to cells of amplifier and press button to continue.

It is recommended to sediment drops from walls of tubes by short vortex (1–3 sec) before their insertion in thermocycler.

Table 1.

Programming thermocyclers at cDNA amplification of *hepatitis A virus*

step	Thermocyclers with active temperature adjustment:								
	"GeneAmp PCR System 2400" (ABI); "Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" (ABI); "Gradient Palm Cycler" (Corbett Research)			"Maxygene" (Axygen)		
	temperature	time	cycles	temperature	time	cycles	temperature	time	cycles
0	95 °C	pause		95 °C	pause		95 °C	pause	
1	95 °C	5 min	1	95 °C	5 min	1	95 °C	5 min	1
2	95 °C	10 sec	42	95 °C	30 sec	42	95 °C	30 sec	42
	67 °C	10 sec		67 °C	30 sec		67 °C	45 sec	
	72 °C	10 sec		72 °C	30 sec		72 °C	45 sec	
3	72 °C	1 min	1	72 °C	1 min	1	72 °C	1 min	1
4	10 °C	storage		4 °C	storage		4 °C	storage	

[REF](#) V4-50-R0,5-CE or V4-50-R0,2-CE

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Programming thermocyclers at cDNA amplification of *hepatitis A virus*

Thermocyclers with block temperature adjustment: "Uno-2" (Biometra), "MiniCycler", "PTC-100"(MJ Research)			
step	temperature	time	cycles
0	95 °C	pause	
1	95 °C	5 min	1
2	95 °C	1 min	42
	67 °C	1 min	
	72 °C	1 min	
3	72 °C	1 min	1
4	4 °C	storage	

Amplification in thermocycler with block temperature adjustment lasts 2 h, in thermocycler with active temperature adjustment — 1 h 30 min.

After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis.

Analysis of amplification products is performed by separation of cDNA fragments in agarose gel.

The amplified samples can be stored for 16 h at room temperature, for 1 week at 2 – 8 °C (be sure to warm the samples to room temperature before running electrophoresis).

8. DATA ANALYSIS

We recommend the following detection agarose kit:

- "EPh" variant 200, [REF](#) K5-200-CE.

Analysis of results is based on the presence or absence of specific bands of amplified cDNA in agarose gel (1.7%). The length of specific amplified cDNA fragments is:

- **HAV** - 290 bp
- **IC HAV-rec** - 560 bp



Put the protective mask or use the glass filter while watching and photographing the gel

8.1. Results interpretation

Table 2.

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel		Interpretation
		290 bp	560 bp	
PC	RNA isolation	Yes	Yes	Valid result
C-	RNA isolation	No	Yes	Valid result
NCA	Amplification	No	No	Valid result
C+	Amplification	Yes	No	Valid result

- The sample is considered to be positive for *hepatitis A virus* RNA if the band of 290 bp is present in agarose gel. The band of IC (560 bp) could be absent in the samples with high concentration of *HAV* RNA.
- The sample is considered to be negative for *hepatitis A virus* RNA if the band of 290 bp is absent and the band of 560 bp is present. Besides specific bands the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 bp of nucleotide pairs.

9. TROUBLESHOOTING

Results of analysis are not being registered in the following cases:

- If results of control points analysis do not correspond to the listed above (Table 2), then the tests are to be re-installed. Discard any reagents that may be suspect.
- If in lanes none of bands of 290 and 560 nucleotide pairs is observed, result of analysis for this sample is irrelevant and investigation of this sample must be repeated from the very beginning. It can be caused by mistake in clinical processing that provoked

loss of RNA/DNA or inhibition of RT and/or PCR.

- If in lines nonspecific bands at different levels are presented, it may be caused by lack of "hot start" or false temperature regimen in thermocycler.
- If in lanes corresponding to negative control (NCA, C-) specific band of 290 bp appears, it means that reagents or samples contamination has taken place. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting contamination source must be undertaken.

10. STABILITY AND STORAGE

The all components of the AmpliSens® *HAV*-EPh PCR kit should be stored from 2 °C to 8 °C and are stable until the expiry date stated on the label.

11. SPECIFICATIONS

11.1. Sensitivity

Analytical Sensitivity of AmpliSens® *HAV*-EPh PCR kit is no less than 1x10³ copies of *Hepatitis A virus* RNA per 1 ml of plasma sample and no less than 5x10³ copies of *Hepatitis A virus* RNA per 1 ml of fecal or water sample.



Claimed analytical features of AmpliSens® *HAV*-EPh PCR kit are guaranteed only when additional kits of reagents, "RIBO-sorb" or "RIBO-prep", "REVERTA-L", and "EPh", are used.

11.2. Specificity

Specificity of AmpliSens® *HAV*-EPh PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.

12. REFERENCES

1. Benjamin RJ. Nucleic acid testing: update and applications. *Semin Hematol.* 2001 Oct; 38 (4 Suppl 9):11-6.
2. Handbook "Sampling, transportation, storage of clinical material for PCR diagnostics", developed by Federal State Institution of Science "Central Research Institute of Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

13. QUALITY CONTROL

In accordance with Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485 –certified Total Quality Management System, each lot of AmpliSens® *HAV*-EPh PCR kit is tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS



Manufacturer



Temperature limitation



Use by



Batch code



For *in Vitro* Diagnostic Use



Version



Catalogue number



Internal Control complex



Contains sufficient for <n> tests



Authorized representative in the European Community.



Consult instructions for use



Caution, consult accompanying documents



For working with Rotor-Gene™ 3000/6000



For working with iQ5, iQ iCycler



Positive control



Negative control