Virus Hepatitises
**Reagent Kits Format and Composition**

**By detection type:**

- **FRT format – real-time fluorescence detection**
  
  The format is intended for use of specialized equipment for real-time PCR. Labeling of reagent kits reflects the adapted equipment:

  - RG — Rotor-Gene 3000/6000 (Corbett Research)
  - IQ — iCycler/iQ5 (BioRad)
  - Mx — Mx3000P/Mx3005P (Stratagene)

- **FEP format – end-point fluorescence detection**

  The format is intended for amplification in a standard thermal cycler with subsequent detection of the end point fluorescent signal on a specialized fluorescent detector, for example, ALA-1 (BioSan), Gene (DNA-Technology) or a real-time PCR unit with detection of fluorescence end point, for example, Rotor-Gene 6000 (Corbett Research).

- **EPh format – electrophoretic detection**

  The format is intended for detection with use of electrophoresis in agarous gel.

**By configuration:**

- **Complete Set Reagent Kit format**

  The kit includes reagents for extraction, amplification and detection.

- **Amplification Reagent Kit (PCR Kit) format**

  The kit includes only amplification reagents.

- **Reverse Transcription and Amplification Reagent Kit format**

  The kit includes reagents for reverse transcription and amplification.

**By hot start type and filling:**

- **“Wax” format**

  Hot start is ensured by a wax layer.

  The kit includes PCR test tubes ready for use with a lower mixture applied under wax.

  The kit includes vials with reagents not dispensed into PCR test tubes.

- **“Hot-Start” format**

  “Hot Start” is ensured by modified polymerase activated at heating (TaqF).

  The kit includes vials with reagents not dispensed into PCR test tubes, modified TaqF polymerase is used.

  As compared to PCR test tubes ready for use with a lower mixture applied under wax this format improves the quality of the “hot start” and quality of results without increasing the associated labour intensity. On preparation to PCR test all components are pre-mixed and then the reaction mixture is dispensed into PCR test tubes once.

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**Reagent Kits for Reverse Transcription and Amplification**

- **«RIBO-sorb» Reagent Kit for RNA/DNA nucleic acids isolation**
  - Lysing solution
  - Sorbent (silica)
  - Washing solutions
  - Eluting solution

- **Electrophoresis (EP) Reagent Kit**
  - TBE-buffer with ethidium bromide
  - Agarose

- **Reagent Kits for Reverse Transcription and Amplification**
  - PCR-mixture-2 (a mixture of the buffer solution and non-modified polymerase)
  - PCR-mixture-1 (primers) in a single test tube or applied under wax in test tubes for PCR 0.5 ml (R0.5) or 0.2 ml (R0.2)
  - Positive control sample (PCS) of the isolation stage and PCR
  - Negative control sample (NCS) of the isolation stage and PCR
  - Internal control sample (ICS) of the isolation stage and PCR
  - Mineral oil

- **Reverta-L Reverse Transcription Reagent Kits**
  - M-MLV reverse transcriptase
  - A revertase buffer for reverse transcription with random-primers
  - RTG-mix reverse transcription facilitator
Virus Hepatitises

Virus hepatitises are a group of infectious liver diseases provoked by hepatotropic viruses belonging to different families. There are 5 major viruses that cause virus hepatitises. They make up two groups of hepatitises: enteric (HAV and HEV) and parenteral (HBV, HCV and HDV) (Table 1). Enteric hepatitises are characterized by a fecal-oral transmission way and these viruses cause only acute hepatitis. Viruses of enteric hepatitises possess high infectivity and stability. Viruses of hepatitises B, C and D are enveloped in a membrane, are transmitted by parenteral way and are able to promote not only acute but chronic virus hepatitis. Viruses of hepatitis B and C play an important role in development of chronic virus liver diseases, they are responsible for development of 60-70 percent of hepatic cirrhosis and up to 70-80 percent of primary liver cancers. Due to a great incidence of virus hepatitises one of the major tasks is development of highly sensitive and reproducible methods of diagnostics allowing detection of the causative agent at all stages of the disease as well as monitoring of antiviral therapy effectiveness.

Table 1. Major human hepatitis viruses

<table>
<thead>
<tr>
<th>Causative agent</th>
<th>Family</th>
<th>Genome</th>
<th>Major transmission way</th>
<th>Chronization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis A Virus (HAV)</td>
<td>Picornaviridae</td>
<td>RNA, 7500 nc</td>
<td>fecal-oral</td>
<td>no</td>
</tr>
<tr>
<td>Hepatitis B Virus (HBV)</td>
<td>Hepadnaviridae</td>
<td>DNA, 3200 pn</td>
<td>parenteral</td>
<td>yes</td>
</tr>
<tr>
<td>Hepatitis C Virus (HCV)</td>
<td>Flaviviridae</td>
<td>RNA, 9500 nc</td>
<td>parenteral</td>
<td>yes</td>
</tr>
<tr>
<td>Hepatitis D Virus (HDV)</td>
<td>Deltavirus (viroid)</td>
<td>PHK, 1700 нк</td>
<td>parenteral</td>
<td>yes</td>
</tr>
<tr>
<td>Hepatitis E Virus (HEV)</td>
<td>Calicivirus</td>
<td>PHK, 7500 нк</td>
<td>fecal-oral</td>
<td>no</td>
</tr>
</tbody>
</table>

Hepatitis А Virus (HAV)

Laboratory diagnostics of Hepatitis A Virus

So far, the enzyme-linked immunosorbent analysis (ELISA) has been extensively used for detection of hepatitis A virus specific markers of IgM and IgG class. Production of antibodies of IgM class (aHAV IgM) starts at the end of the incubation period that lasts for 30 days at the average. Their detection allows identification of the asymptomatic infection, which has practical importance. But test for aHAV IgM might produce falsely positive results (5 percent of cases). The synthesis of specific antibodies of IgG (aHAV IgG) class starts at the end of the first – beginning of the second week of the disease. They are markers of the hepatitis A patient. They had in the past or the evidence of the post-vaccination immunity. To diagnose acute forms of HAV this serological marker is of no importance. The virus antigen (HAV-Ag) is not a diagnostic marker as it circulates in blood only for a short period of time. It’s detected in faeces in the incubation period and, as a rule, within the first two-three weeks of the disease. This marker is also used for detection of hepatitis A virus in environmental entities.

Molecular Biological Methods

Possessing a higher sensitivity, these methods have been extensively used in diagnostics and study of the hepatitis A virus. Detection of the causative agent RNA by PCR method has significant advantages as related to ELISA and biochemical tests at detection of the virus in blood of contact persons as RNA of the hepatitis A virus manifests itself in the blood on the third week from the moment of contamination and is detected at the average within 20 days after appearance of the disease symptoms. Thus, RNA is the first diagnostic marker detected in the patient blood, occurs earlier than aHAV IgM and gives no falsely negative reactions (Figure 1). Detection of RNA of the hepatitis A virus with the help of PCR in faeces is possible from the third week of the incubation period and up to three months after manifestation of the disease symptoms. Detection of RNA has more advantages (by 1000 times more at the least) as compared to detection of HAV-Ag in environmental entities (drinking or waste waters, waters from impounded surface waters and so on).

Passport of Hepatitis A Virus (HAV)

The virus hepatitis A (HAV) is the enteric infection most widely spread in the world. This is the acute infectious disease of liver transmitted by fecal-oral way, the causative agent of which is hepatitis A virus (HAV) belonging to the family Picornaviridae. Virus hepatitis A is one of the five most economically significant infectious diseases and one of the priority problems of the public healthcare. HAV is characterized by a nonpercutaneous-social channel of infection provoking sporadic cases of contamination whereas food and water transmission ways promote outbursts of the disease. The epidemiological situation as concerning HAV in Russia remains unfavorable, the highest incidence of the disease being in regions with serious problems of high quality drinking water supply to population and drainage systems. The recent years saw increase of the number of HAV outbursts.

Solution of diagnostic and epidemiological aspects of the hepatitis A issue is to a large degree determined by possibilities of methodic approaches.

Figure 1. Dynamics of markers and duration of viremia in acute virus hepatitis A (Bower W.A., Nainan O.V., Han X. et al. Duration of Viremia in Hepatitis A Virus Infection. J Inf Dis, 2000; 182: 12-17)
Reagent kits for detection of hepatitis A virus (HAV) and hepatitis E virus (HEV) RNA

Representative works. Eph format.

Dilutions of the control sample

1 - Hepatitis A virus strain (no dilution);
2-8 - ten-fold dilutions of the basic Hepatitis A virus strain (from $10^{-1}$ to $10^{-7}$, respectively);
9 - NCS (negative control sample);
10 - PCS (positive control sample (PCS HAV-rec));
11 - C- (negative control sample (PCR stage));
12 - C+ (positive control sample PCS cDNA HAV);
M - marker of molecular weight.

Clinical samples

1, 3, 5, 7, 9, 11 - HAV-positive assays;
2, 4, 6, 8, 10 - HAV-negative assays;
13 - NCS (negative control sample);
14 - C+ (positive control sample PCS cDNA HAV);
M - marker of molecular weight.

Explanation:

Configuration of kits:
- a "complete set reagent kit" includes reagents for extraction, amplification and detection;
- an "amplification reagent kit" (PCR-set) includes only amplification reagents.
- a "reverse transcription and amplification reagent kit"

Kit types:
"Hot Start" is provided by a wax layer;
- a set includes ready to use PCR test tubes with the lower mixture applied under the wax

"Hot Start" is provided by a modified polymerase (TaqF) activated at heating:
- a set includes vials with reagents not dispensed into PCR test tubes, a modified polymerase TaqF is used.

Advantages of reagent kits

- Use of recombinant internal control samples allows control of all stages of analysis (RNA isolation, reverse transcription and PCR) and evaluation of PCR inhibitors effect on results of the test.
- Detects all genotypes of hepatitis A that might be present in humans.
- Allows detection of hepatitis A virus RNA in clinical material and environmental entities.
- Convenient, quick and cheap PCR format.

Clinical material for examination

<table>
<thead>
<tr>
<th>Type of clinical material</th>
<th>Recommended kits for extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma (serum) of peripheral blood</td>
<td>RIBO-sorb  RIBO-Prep</td>
</tr>
<tr>
<td>Faeces</td>
<td>RIBO-sorb</td>
</tr>
<tr>
<td>Concentrates (eluates) of water assays</td>
<td>RIBO-sorb</td>
</tr>
<tr>
<td>Concentrates (eluates) of lavage from food products</td>
<td>RIBO-sorb</td>
</tr>
</tbody>
</table>

- a kit is included in the complete set reagent kit

Instrumentation for collection of material

We recommend blood collection with use of vacuum systems (vacuum test tubes with EDTA-K3, Green Vacutube cat. No.GV0414 or Vacuette cat. No.454039).

We recommend faeces collection in the 60 ml plastic container with a spoon (cat. No. ILS-KPL-60, cat No. ILS-KPL-60C).

Variants of Reagent Kits. Eph Format – Electrophoretic Detection

Attention! The technology presents danger of contamination! A separate room and personnel are required for the detection!

<table>
<thead>
<tr>
<th>Cat.No.</th>
<th>Name</th>
<th>Set</th>
<th>No. of tests</th>
<th>Type</th>
<th>Special equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV4-50-R0.5</td>
<td>AmpliSens® HAV-EPH</td>
<td>📍</td>
<td>50</td>
<td>📌</td>
<td>Electrophoretic chamber, gel-documentation system</td>
</tr>
<tr>
<td>TV4-50-R0.2</td>
<td>AmpliSens® HAV-EPH</td>
<td>📍</td>
<td>50</td>
<td>📌</td>
<td></td>
</tr>
<tr>
<td>V4-50-R0.5</td>
<td>AmpliSens® HAV-EPH</td>
<td>📍</td>
<td>55</td>
<td>📌</td>
<td></td>
</tr>
<tr>
<td>V4-50-R0.2</td>
<td>AmpliSens® HAV-EPH</td>
<td>📍</td>
<td>55</td>
<td>📌</td>
<td></td>
</tr>
</tbody>
</table>

Analytical properties

- Sensitivity: 5 x10^3 GE per ml in water and faeces samples, 1 x10^2 GE per ml in blood plasma samples
- Specificity: No cross reactions for viruses of hepatitis B, C, Delta, G and E; as well as enteric virus strains (Coxsakie B1, B2, B3, B4, B5, B6, Polio I, II, III), respiratory viruses (adenoviruses of serogroups 5 and 7; viruses of A-type flu), WA human rotavirus, astroviruses, noroviruses of types I and II, microorganisms of Shigella, Salmonella, Yersinia, Campylobacter families.
Results of clinical tests

Sensitivity and specificity of the test were evaluated in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute as compared to ELISA test systems for detection of HAV-antigen and antibodies. The experimental group included 60 patients with acute viral hepatitis A. The experimental group included 60 patients without manifestations of acute viral hepatitis A, patients with acute or chronic hepatitis of other etiologies. The diagnostic specificity for plasma blood samples made 100 percent, the diagnostic sensitivity – 99.5 percent; for feces the diagnostic specificity made 100 percent; the value of diagnostic sensitivity reached 100 percent. Experiments on model experimental samples (strain of HAS-15 of hepatitis A virus) were carried out on a series of ten-fold dilutions of the virus in tap water and water from open surface waters 106-101 TCD per ml. In the course of testing it was shown that the experimental sensitivity of hepatitis A virus detection method based on PCR exceeds by 10,000 times the sensitivity of ELISA by detection of HAV-antigen.

Representative works. FRT format

![Fluorescence Detection in Real-Time Regime](since Q4 Y2009)

<table>
<thead>
<tr>
<th>Cat.No.</th>
<th>Name</th>
<th>Set</th>
<th>No. of tests</th>
<th>Type</th>
<th>Mark</th>
<th>Special equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR-V4 (RG)</td>
<td>AmpliSens® HAV-FL</td>
<td>50</td>
<td>FAM/Green, JOE/Yellow</td>
<td>Rotor-Gene 3000/6000 (Corbett Research)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-V4 (RG)</td>
<td>AmpliSens® HAV-FL</td>
<td>55</td>
<td>FAM/Green, JOE/Yellow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>AmpliSens® HEV-FL</td>
<td>50</td>
<td>FAM/Green, JOE/Yellow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>AmpliSens® HEV-FL</td>
<td>55</td>
<td>FAM/Green, JOE/Yellow</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analytical properties

Sensitivity 500 GE/ml

Specificity

No cross reactions for viruses of hepatitis B, C, Delta, G and A (for the HEV kit), E (for HAV kit), as well as enteric virus strains (Coxsakie B1, B2, B3, B4, B5, B6, Polio I, II, III), respiratory viruses (adenoviruses of serogroups 5 and 7; viruses of A-type flu), WA human rotavirus, astroviruses, noroviruses of types I and II, microorganisms of Shigella, Salmonella, Yersinia, Campylobacter families.

![End Point Fluorescence Detection](since Q4 Y2009)

<table>
<thead>
<tr>
<th>Cat.No.</th>
<th>Name</th>
<th>Set</th>
<th>No. of tests</th>
<th>Type</th>
<th>Mark</th>
<th>Special equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR-V4-FEP</td>
<td>AmpliSens® HAV-FEP</td>
<td>50</td>
<td>Fam/Hex</td>
<td>Two-channel and more PCR detectors: Jin (DNA-Technology), ALA-1 (BioSan)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-V4-FEP</td>
<td>AmpliSens® HAV-FEP</td>
<td>55</td>
<td>Fam/Hex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>AmpliSens® HEV-FEP</td>
<td>50</td>
<td>Fam/Hex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>AmpliSens® HEV-FEP</td>
<td>55</td>
<td>Fam/Hex</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analytical properties

Sensitivity 500 GE/ml

Specificity

No cross reactions for viruses of hepatitis B, C, Delta, G and A (for the HEV kit), E (for HAV kit), as well as enteric virus strains (Coxsakie B1, B2, B3, B4, B5, B6, Polio I, II, III), respiratory viruses (adenoviruses of serogroups 5 and 7; viruses of A-type flu), WA human rotavirus, astroviruses, noroviruses of types I and II, microorganisms of Shigella, Salmonella, Yersinia, Campylobacter families.
Hepatitis C Virus (HCV)

Passport of hepatitis C virus (HCV)

Hepatitis C virus (HCV) is RNA-containing, hepatotropic virus belonging to the Flaviviridae family. Contamination with hepatitis C virus occurs at direct entering of the virus in blood (at parenteral interventions or during hemotransfusions). Due to a limited number of symptoms of the acute period of the hepatitis C virus (HCV) the disease in most cases proceeds unperceived by the patient and attending physicians and the frequency of chronicization makes 80 percent. Chronic viral hepatitis C (CHC) is one of the most widely spread diseases that affect liver and lead to cirrhoses and hepatocellular carcinoma (HCC). At present there are more than 170 million of infected people, which makes up 3 percent of the population of the world. On the background on constant increase of the number of infected persons and lack of the vaccine the urgency of CHC is increasing with each year. Results of laboratory experiments play an important role for establishment of the fact of contamination, determination of indications to antiviral therapy; choice of the optimum therapeutic regime; timely evaluation of the effectiveness of the therapy and assessment of the persistent response to treatment.

Laboratory Diagnostics of Viral Hepatitis C

Serological Diagnostics

Major serological markers of the viral hepatitis C are anti-HCV IgM and anti-HCV IgG. Anti-HCV IgM — antibodies to HIV antigens of IgM class are a marker of acute HCV-infection. But a number of experimental works produce evidence that they might be lacking in acute infection and develop after antibodies of IgG class and persist for a long time. In addition to this, antibodies of this class are observed in a great number of cases of chronic HCV-infection. Anti-HCV IgG — antibodies to HCV antigens of IgG class are detected in 8-12 weeks after contamination with HCV. But presence of antibodies of this class doesn’t evidence the continuing replication of the virus and might be a manifestation of the current as well as of the previous disease.

Antibodies develop to each of HCV proteins. HCV screening requires use of ELISA method. There are three generations of ELISA test systems that differ from each other by sensitivity and specificity.

Molecular -Biological Diagnostics Methods

The leading position in laboratory diagnostics of HCV is taken by molecular-biological methods allowing: 1) determination of the contamination fact; 2) establishment of indications to antiviral therapy; 3) choice of the optimum therapeutic regime; 4) timely evaluation of the effectiveness of the therapy and 5) assessment of the persistent response to treatment.

Diagnostic Importance of Quality Methods of HCV RNA Detection

- Detection of HCV RNA with the help of molecular-biological methods (with use of PCR) is used for early diagnostics of the acute viral hepatitis C. HCV RNA appears in blood plasma in 3 days after introduction of infection. In patients HCV RNA in detectable levels is found in blood plasma on the average by the 11th day after contamination (Figure 3). This allows diagnostics of the acute hepatitis C long before seroconversion, which is especially important for the early diagnostics and testing of donor blood.
- Effectiveness of the antiviral therapy is assessed by qualitative detection of HCV RNA in blood plasma in the course of the therapy and on its termination. This is achieved through qualitative test systems with high sensitivity (50 IU per ml) (International Consensus on HCV Treatment, 2002, Figure 2).
- Assessment of the persistent response to therapy is carried out by qualitative detection of HCV RNA in blood plasma in 24 weeks after termination of the therapy. This is achieved through qualitative test systems with high sensitivity (50 IU per ml) (International Consensus on HCV Treatment, 2002, Figure 2).

Diagnostic importance of genotyping of hepatitis C virus

The most important factor on which the effectiveness of the antiviral therapy depends is genotype of the hepatitis C virus. In accordance with the modern classification HCV is subdivided in 6 genotypes, each of which is subdivided in its turn in several subtypes. The virus genotype is designated by Arab figures and the subtype - by small Latin letters. A number of investigatory works provides that HCV Genotype 1 is less responsive to antiviral therapy as compared to other virus genotypes. This evidence has served a basis for development of various recommendations on treatment of patients with Genotype 1 and Genotypes 2 and 3 and was recorded in the consensus opinion of the International Experts of HCV Treatment (Figure 2). Publications include scarce information of genotypes 4, 5 and 6, though these genotypes occur in regions with high incidence of HCV. Genotype 4 prevails in Egypt and Africa, Genotype 5 – in South Africa, Genotype 6 – in the South-Eastern Asia. Thus, HCV genotype is an important factor determining tactics of the antiviral therapy and its effectiveness.
In spite of the fact that the majority of researches produce evidence that the level of the liver affection in HCV doesn't depend on the viral load, patients with a high viral load before treatment are less responsive to antiviral therapy by interferon preparations. The low viral load (<600,000 IU per ml) is an indispensable factor affecting the persistent virusological response. A number of works show that patients with Genotype 3 and high viral load (>600,000 IU per ml) are less responsive to therapy as compared to patients with Genotype 2 or Genotype 3 coupled with low viral load and possible require a longer course of treatment.

Quantitative evaluation of HCV RNA level in blood plasma at antiviral therapy of patients infected with 1st genotype HCV is used for monitoring of effectiveness of treatment, which is recorded in the consensus opinion of the International Experts of HCV Treatment (Figure 2).

**Collection of Material for Examination of Viruses of Hepatitises B, C and D**

We recommend blood collection with use of vacuum systems with EDTA (vacuum test tubes with EDTA-K3, Green Vac-Tube, 4 ml, 13x75 mm, Green Cross (Korea), cat. No.GV0414, two-way needle for Green Vac-Tube vacuum test tubes, 38 x 0.8 mm, 21Gx1 1/2", cat. No.3021, or Vacuette test tubes with EDTA-K3, 4.5 ml 13x75 mm, violet, Greiner Bio-one (Austria), cat. No.454039, Vacuette needle, 38x0.8 mm, 21Gx11/2, Greiner Bio-one (Austria), cat No.450076).

Blood plasma should be collected by centrifuging of test tubes with whole blood at 800-1600 g (3 thousand rpm) for 20 minutes at room temperature.
Reagent kits for detection of hepatitis C virus (HCV) RNA

The reagent kits are adapted for automatic isolation of nucleic acids on easyMAG unit (BioMerieux).

Advantages of reagent kits

- Use of the recombinant internal control sample allows control of all stages of analysis (RNA isolation, reverse transcription and PCR) and evaluation of PCR inhibitors effect on results of the test.
- Convenient and cheap PCR format.

Clinical material for examination

<table>
<thead>
<tr>
<th>Type of clinical material</th>
<th>Recommended kits for extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma of peripheral blood</td>
<td>RIBO-sorb, RIBO-Prep, NucliSENS reagents for easyMAG</td>
</tr>
</tbody>
</table>

![Image: Representative works. FRT format](image)

Advantages of the format

- Reactions of reverse transcriptase of isolated RNA and amplification to DNA are conducted in a single reaction buffer with the help of the MMLV reverse transcriptase and Taq-polymerase.
- Higher effectiveness of detection and lower labour intensity of detection.
- Reagent kits are adapted for NucliosSENS easyMAG and a wide range of units for PCR in the real-time regime.
- Maximum test sensitivity.

Analytical properties

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>100 IU per ml, up to 10 IU per ml at use of the easyMAG unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>No cross reactions for adenoviruses (types 2, 3 and 7), cytomegalovirus, Epstein-Barr virus, chicken pox virus, hepatitis B virus, human immunodeficiency virus of type 1, human herpes virus, types 6 and 8, simplex herpes virus, types 1, 2; HPV types 6, 11, 16, 18, 33, 35.</td>
</tr>
</tbody>
</table>

Results of clinical tests

Sensitivity and specificity of the test were evaluated in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute as compared to “AmpliSens® HCV” for detection of HCV-virus RNA by PCR method with electrophoretic detection of amplification products in the agarous gel (FSP 42-0155-1135-0). The reagent kit was tested on clinical samples (blood plasma) and dilutions of the negative reference sample (NRS) HCV RNA (NRS 42-28-366-06P). The experimental group included 52 patients with positive tests by ELISA for antibodies to hepatitis C virus and 5 dilutions of NRS HCV RNA. The total number of samples made 57. The control group was represented by blood plasma samples of patients with hepatitises of other etiologies and samples of healthy persons-donors. The total number of samples made 30. The diagnostic specificity of the tested kit made 100 percent, the diagnostic sensitivity – 100 percent.
Attention! The technology presents danger of contamination! A separate room and personnel are required for the detection!

Analytical properties

<table>
<thead>
<tr>
<th>Cat.No.</th>
<th>Name</th>
<th>Set</th>
<th>No. of tests</th>
<th>Type</th>
<th>Special equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV1-R0.5-FEP</td>
<td>AmpliSens® HCV-FEP</td>
<td>48</td>
<td></td>
<td>FamHex</td>
<td>Detectors ALA-1 (BioSan), Gene (DNA-Technology)</td>
</tr>
<tr>
<td>TV1-R0.2-FEP</td>
<td>AmpliSens® HCV-FEP</td>
<td>48</td>
<td></td>
<td>FamHex</td>
<td>Detector ALA-1 (BioSan)</td>
</tr>
<tr>
<td>V1-R0.5-FEP</td>
<td>AmpliSens® HCV-FEP</td>
<td>48</td>
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<td>FamHex</td>
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<td>V1-R0.2-FEP</td>
<td>AmpliSens® HCV-FEP</td>
<td>48</td>
<td></td>
<td>FamHex</td>
<td>Detector ALA-1 (BioSan)</td>
</tr>
<tr>
<td>V1-100-R0.5-FEP</td>
<td>AmpliSens® HCV-FEP</td>
<td>96</td>
<td></td>
<td>FamHex</td>
<td>Detector ALA-1 (BioSan)</td>
</tr>
<tr>
<td>V1-100-R0.2-FEP</td>
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<td>96</td>
<td></td>
<td>FamHex</td>
<td>Detector ALA-1 (BioSan)</td>
</tr>
</tbody>
</table>

Results of clinical tests

Sensitivity and specificity of the test were evaluated in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute as compared to “AmpliSens® HCV” for detection of HCV-virus by PCR method with electrophoretic detection of amplification products in the agarose gel (FSP 42-0155-1135-0). The reagent kit was tested on clinical samples (blood plasma) and dilutions of the negative reference sample (NRS) HCV RNA dilutions (NRS 42-28-366-06P). The experimental group included 52 patients with positive tests by ELISA for antibodies to hepatitis C virus. The control group was represented by blood plasma samples of patients with hepatitis C and samples of healthy persons-donors. The total number of samples made 30. The diagnostic specificity of the tested kit made 100 percent, the diagnostic sensitivity – 100 percent.

Representative works. Eph format

2, 3, 6, 9 - HCV-positive assays;
1, 4, 5, 7, 8, 10-13 - HCV-negative assays;
14 - NCS (negative control sample);
15 - C- (negative control sample (PCR stage));
16 - C+ (positive control sample PCS cDNA HCV);
M - marker of molecular weight.

Explanation:

Configuration of kits:

- a “complete set reagent kit” includes reagents for extraction, amplification and detection;
- an “amplification reagent kit” (PCR-set) includes only amplification reagents;
- a “reverse transcription and amplification reagent kit”

Kit types:

“Hot Start” is provided by a wax layer:
- a set includes ready to use PCR test tubes with the lower mixture applied under the wax
- a set includes vials with reagents not dispensed into PCR-test tubes.
“Hot Start” is provided by a modified polymerase (TaqF) activated at heating:
- a set includes vials with reagents not dispensed into PCR test tubes, a modified polymerase TaqF is used.

Results of clinical tests

Sensitivity and specificity of the test were evaluated in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute as compared to ELISA test-systems for detection of anti-HCV-Ab and anti-HCV-IgM-Ab. The reagent kit was tested on clinical samples (blood plasma) and various dilutions of the standard NIBSC HCV RNA PCR 98/576. The experimental group included 37 patients with the diagnosis virus hepatitis C and 25 various dilutions of NIBSC HCV RNA PCR 98/576 standard. The total number of samples made 62. The control group was represented by blood plasma samples of patients with hepatitis C and samples of healthy persons-donors (34 samples), samples of bovine fetal serum (20 samples) and samples of HCV-negative plasma (11 samples). The total number of samples made 145. The value of diagnostic specificity of the tested kit made 97.2 percent, the diagnostic sensitivity – 97.9 percent.
Reagent kits for detection of hepatitis C virus (HCV) genotype in clinical material

Representative works. FRT format

AmpliSens® HCV-genotype-FRT

Data by FAM/Green channel (detection of 1a, 1b subtypes of HCV and internal control – 3 test tubes)

AmpliSens® HCV-genotype-1/2/3-FRT

Data by JOE/Yellow channel (detection of 3a, 2, 4 genotypes of HCV – 3 test tubes)

AmpliSens® HCV-genotype-1 (RG, IQ)

Data by FAM/Green channel (genotype 1)

AmpliSens® HCV-genotype-2 (RG, IQ, Mx)

Data by ROX/Orange channel (genotype 2)

AmpliSens® HCV-genotype-3 (RG, IQ, Mx)

Data by Cy5/Red channel (detection of IC)

AmpliSens® HCV-genotype

Data by FAM/Green channel (detection of 1a, 1b subtypes of HCV and internal control – 3 test tubes)

Data by JOE/Yellow channel (detection of 3a, 2, 4 genotypes of HCV – 3 test tubes)

Data by ROX/Orange channel (genotype 3 HCV)

Data by Cy5/Red channel (detection of IC)

AmpliSens® HCV-genotype-1/2/3-FL

Data by FAM/Green channel (genotype 1HCV)

Data by JOE/Yellow channel (genotype 2HCV)

Data by ROX/Orange, Cy5/Red channel (detection of IC)

AmpliSens® HCV-genotype-1/2/3-FL

Data by FAM/Green channel (genotype 1)

Data by JOE/Yellow channel (genotype 2)

Data by ROX/Orange channel (genotype 3)

Data by Cy5/Red channel (detection of IC)

FRT format – Fluorescence Detection in Real-Time Regime

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Name</th>
<th>Set</th>
<th>No. of tests</th>
<th>Type</th>
<th>Mark</th>
<th>Special equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR-V1-G-2x (RG, IQ)</td>
<td>AmpliSens® HCV-genotype</td>
<td>48</td>
<td>FAM/Green, JOE/Yellow</td>
<td>Two- and more channel units Rotor-Gene 6000 (Corbett Research), IQ iCycler, iQ5 (BioRad)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-V1-G-4x (RG, IQ, Mx)</td>
<td>AmpliSens® HCV-1/2/3-FL</td>
<td>120</td>
<td>FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red</td>
<td>Four- and more channel units Rotor-Gene 6000 (Corbett Research), IQ iCycler, iQ5 (BioRad), Mx3000P/Mx3005P (Stratagene)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The AmpliSens® HCV-1/2/3-FL reagent kit allows detection and differentiation of 1, 2 and 3 HCV genotypes in a single test tube (a four-channel unit is required), the AmpliSens® HCV-genotype reagent kit allows detection and differentiation of genotypes 1 (subtypes 1a and 1b separately), 2, 3 and A of HCV in 3 test tubes (a two-channel unit is required).

Analytical properties

Sensitivity

AmpliSens® HCV-genotype — 1000 IU per ml
AmpliSens® HCV-1/2/3-FL — 500 IU per ml

Specificity

No cross reactions for genotypes 1, 2, 3, 4 of hepatitis C virus, for genotypes 5 and 6 of hepatitis B virus, human immunodeficiency virus of type 1; human herpes virus, types 6 and 8; simplex herpes virus, types 1, 2; HPV types 6, 11, 16, 18, 33, 35.

Results of clinical tests

The AmpliSens® HCV-genotype reagent kit was tested on clinical samples (blood plasma). The experimental group included 230 patients with the diagnosis hepatitis C virus represented by various genotypes (1a (43 samples), 1b (59 samples), 3a (60 samples), 2 (54 samples), 4 (4 samples), 5 (3 samples), 6 (1 sample), 1a+1b (6 samples)). The comparative system was represented by AmpliSens® HCV and sequence analysis (for assays not serologically defined by AmpliSens® HCV). The control group was represented by blood plasma samples of patients with hepatitis C virus. The total number of samples made 30. The diagnostic specificity of the tested kit made 100 percent, the diagnostic sensitivity – 100 percent.

The AmpliSens® HCV-genotype-1/2/3-FL reagent kit was tested on clinical samples (blood plasma). The experimental group included 88 patients with the diagnosis chronic viral hepatitis C represented by various genotypes (1 (40 samples), 2 (18 samples), and 3 (30 samples)). The comparative system was represented by sequence analysis. The control group was represented by blood plasma samples of patients with acute viral hepatitis B (40 samples) and samples of healthy persons-donors (90 samples). The total number of samples made 130. The diagnostic specificity of the tested kit made 100 percent, the diagnostic sensitivity – 100 percent.

Data by Cy5/Red channel (detection of IC)

- Convenient and quick PCR format.
- No stage of nested PCR.
- Reagent kits detect major genotypes (subtypes) of HCV occurring in the territory of the RF and CIS countries (1a, 1b, 2, 3a, 4). AmpliSens® HCV-genotype can also detect Genotype 4 of HCV.

Clinical material for examination

<table>
<thead>
<tr>
<th>Type of clinical material</th>
<th>Recommended kits for extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma of peripheral blood</td>
<td>RIBO-sorb®</td>
</tr>
</tbody>
</table>

- A kit is included in the complete set of reagent kit (⁎)
**FEP Format. End Point Fluorescence Detection**

<table>
<thead>
<tr>
<th>Cat.No.</th>
<th>Name</th>
<th>Set</th>
<th>No. of tests</th>
<th>Type</th>
<th>Special equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV1-G-FE</td>
<td>AmpliSens® HCV-1/2/3-FL</td>
<td></td>
<td>120</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Analytical properties**

Sensitivity: 500 IU per ml

Specificity: No cross reactions for genotypes 1, 2, 3, 4 of hepatitis C virus, adenoviruses (types 2, 3 and 7), cytomegalovirus, Epstein-Barr virus, chicken pox virus, hepatitis B virus, human immunodeficiency virus of type 1; human herpes virus, types 6 and 8; simplex herpes virus, types 1, 2; HPV, types 6, 11, 16, 18, 33, 35.

**Results of clinical tests**

The AmpliSens® HCV-genotype-1/2/3-FL reagent kit was tested on clinical samples (blood plasma). The experimental group included 88 patients with the diagnosis chronic viral hepatitis C represented by various genotypes (1 (40 samples), 2 (18 samples), and 3 (30 samples)). The comparative system was represented by sequence analysis. The control group was represented by blood plasma samples of patients with acute viral hepatitis B (40 samples) and samples of healthy persons-donors (90 samples). The total number of samples made 130. The diagnostic specificity of the tested kit made 100 percent, the diagnostic sensitivity – 100 percent.

---

**Eph Format - Electrophoretic Detection**

**Attention! The technology presents danger of contamination! A separate room and personnel are required for the detection!**

<table>
<thead>
<tr>
<th>Cat.No.</th>
<th>Name</th>
<th>Set</th>
<th>No. of tests</th>
<th>Type</th>
<th>Special equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV1-G-50-R0.5</td>
<td>AmpliSens® HCV-genotype</td>
<td></td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TV1-G-50-R0.2</td>
<td>AmpliSens® HCV-genotype</td>
<td></td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1-G-50-R0.5</td>
<td>AmpliSens® HCV-genotype</td>
<td></td>
<td>55</td>
<td></td>
<td>Electrophoretic chamber, gel- documentation system</td>
</tr>
<tr>
<td>V1-G-50-R0.2</td>
<td>AmpliSens® HCV-genotype</td>
<td></td>
<td>55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AmpliSens® HCV-genotype reagent kit allows detection and differentiation of 1a, 1b, and 3a subtypes and 2 HCV genotype in two test tubes

**Analytical properties**

Sensitivity: 10^1 IU per ml

Specificity: No cross reactions for genotypes 1, 2, 3 of hepatitis C virus, adenoviruses (types 2, 3 and 7), cytomegalovirus, Epstein-Barr virus, chicken pox virus, hepatitis B virus, human immunodeficiency virus of type 1; human herpes virus, types 6 and 8; simplex herpes virus, types 1, 2; HPV, types 6, 11, 16, 18, 33, 35.

**Results of clinical tests**

Sensitivity and specificity of the test were evaluated in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute. The alternative method to determine genotype of hepatitis C virus in tested samples that was used is method of direct sequence analysis of obtained amplicons. The kit was tested in the clinical samples (blood plasma). The experimental group included 40 patients with the diagnosis viral hepatitis C with positive results of ELISA for antibodies to hepatitis C and positive results for HCV RNA on the unit AmpliSens® HCV (FSP 42-0155-1135-01). The value of diagnostic sensitivity made 98 percent.
**Reagent kit for quantitative detection of hepatitis C virus (HCV) RNA**

The reagent kit is adapted for automatic isolation of nucleic acids on the easyMAG unit (BioMerieux).

### Advantages of reagent kits
- Quantitative measurements in a wide range of concentrations.
- The format of amplification products detection in the real-time regime without opening of test tubes, which reduces the risk of contamination and excludes the necessity to use a separate room of the PCR-laboratory.
- Reactions of reverse transcription of the isolated RNA and amplification of cDNA are conducted in a single reaction buffer with the help of the MMLV reverse transcriptase and Taq-polymerase.
- Use of the recombinant internal control sample allows control of all stages of analysis (RNA isolation and PCR) and evaluation of PCR inhibitors effect on results of the test.
- Simultaneous analysis of the tested sample and quantitatively characterized internal sample allows determination of HCV RNA concentration in the tested sample.
- The reagent kit is adapted for a wide range of units for PCR in the real-time regime.

### Representative works. FRT format

![Graph](image1)

**Explanation:**

**Configuration of kits:**
- a "complete set reagent kit" includes reagents for extraction, amplification and detection;
- an "amplification reagent kit" (PCR-set) includes only amplification reagents.
- a "reverse transcription and amplification reagent kit"

**Kit types:**

"Hot Start" is provided by a wax layer:
- a set includes ready to use PCR test tubes with the lower mixture applied under the wax
- a set includes vials with reagents not dispensed into PCR-test tubes.

"Hot Start" is provided by a modified polymerase (TaqF) activated at heating:
- a set includes vials with reagents not dispensed into PCR test tubes, a modified polymerase TaqF is used.

### Clinical material for examination

<table>
<thead>
<tr>
<th>Type of clinical material</th>
<th>Recommended kits for extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma of peripheral blood</td>
<td>RIBO-sorb, RIBO-Prep, NucliSens reagents for easyMAG</td>
</tr>
</tbody>
</table>

- a kit is included in the complete set reagent kit (easyMAG)

### Analytical properties

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>5 \times 10^2 IU per ml, linear range of measurements: 5 \times 10^2 — 5 \times 10^7 IU per ml. At utilization of easyMAG unit: 50 IU per ml, a linear range of measurements: 50-5 \times 10^7 IU per ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>No cross reactions for adenoviruses (types 2, 3 and 7), cytomegalovirus, Epstein-Barr virus, chicken pox virus, hepatitis B virus, human immunodeficiency virus of type 1; human herpes virus, types 6 and 8; simplex herpes virus, types 1, 2; HPV types 6, 11, 16, 33, 35.</td>
</tr>
</tbody>
</table>

### Results of clinical tests

Sensitivity and specificity of the test were evaluated in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute as compared to AmpliSens® HCV Monitor test-system with hybridization and enzyme detection of amplification products (FSP 42-0115-6363-05) for evaluation of the HCV viral load. The experimental group included 39 patients with positive results by ELISA test for HCV RNA in the test system AmpliSens® HCV (FSP 42-0155-1135-01) — 15 samples. The difference between results (0.5 lg), obtained with two reagent kits (a tested one and the reference one) in the working range of the test system didn’t exceed 0.5 lg in 95 percent of cases, which signifies the comparability of results. The maximum difference between the results was obtained in the first sample and made 0.84 lg. The specificity of two reagent kits made 100 percent. Determination of the viral load in clinical samples from 10 patients of experimental group before start of the therapy and after 12 weeks of treatment was conducted in order to study the possibility of determination of effectiveness of the antiviral therapy. The viral load in 8 out of 10 patients reduced by more than 2 lg.
Hepatitis B Virus (HBV)

Laboratory diagnostics of viral hepatitis B

Molecular - Biological Diagnostics Methods

Diagnostic importance of qualitative methods of HBV DNA detection

Detection of HBV DNA is used for:

- Early diagnostics of acute viral hepatitis B. HBV DNA appears in blood on the average in a month after introduction of infection and is the first diagnostic marker. HBsAg appears in blood of the infected person at least 3 weeks later (Figure 5);
- Detection of latent forms of viral hepatitis B. HBsAg in serum of infected persons is not detected and HBV DNA is detected in blood plasma or liver tissue at use of highly sensitive PCR-kits;
- Detection of mutant strains of hepatitis B virus by HBsAg. Due to mass programs of vaccination the trend to a greater incidence of such strains is observed. It's possible that detection of such strains by ELISA method might not be achieved, whereas it's easily detected by PCR kits;
- Diagnostic importance of qualitative methods of HBV DNA concentration detection in blood plasma;
- Establishment of diagnosis of chronic viral hepatitis B. Persistence of HBsAg for more than 6 months, a higher level of transaminases, histological manifestations of pronounced hepatitis (activity index >4) and concentration of HBV DNA more than 10^5 GE per ml (2x10^4 IU per ml) for HBsAg-positive patients or 10^4 GE per ml for HBsAg-negative patients allow establishment of the “chronic hepatitis B” diagnosis requiring corresponding therapy. A lower concentration of the virus and the normal level of transaminases indicate the inactive carrier state of the virus requiring dynamic observation without the necessity of antiviral therapy;
- Monitoring of effectiveness of the antiviral therapy. The quantitative investigation of DNA of hepatitis B virus in blood plasma is obligatory for determination of the tactics of the antiviral therapy and evaluation of its effectiveness (each 3-6 months). Increase of the viral load per 1 lg from the minimum value is the sign of development of resistance of the virus to this therapeutic drug.
- Monitoring of activity of virus replication in bearers of HBsAg. HBsAg-positive patients whose values do not satisfy all requirements of chronic viral hepatitis B and who have no counter-indications to the antiviral therapy.

the following dynamic examination is recommended:

- HBsAg-positive, HBeAg-positive patients with a normal level of alanintransaminase (ALT) should test level of ALT each 3-6 months, HBeAg should be tested each 6-12 months. At increase of ALT it’s necessary to conduct quantitative determination of HBV DNA. Liver biopsy is recommended for patients whose viral load is more than 10^6 GE per ml and the level of ALT remains on a higher level (but not more than by 2 times from the normal value) within 3-6 months or the viral load is more than 10^6 GE per ml and the patient is older than 40 years. The antiviral therapy is recommended for those patients whose viral load is more than 10^5 GE per ml and the level of ALT remains increased (by more than 2 times from the normal) within 3-6 months.
- HBsAg-positive, HBeAg-negative patients with a normal level of ALT and a low viral load (<10^4 GE per ml) should test their ALT level each 3 months for one year and each 6-12 months in subsequent years. If the level of ALT is increasing (but not more than by 2 times from the normal value) determination of the viral load is recommended. Liver biopsy is recommended for patients whose viral load makes 10^4 -10^5 GE per ml and the level of ALT remains on a higher level (but not more than by 2 times from the normal value) within 3 months. If results of the liver biopsy show moderate/grave inflammation or significant fibrosis, these patients should start antiviral therapy. The antiviral therapy is recommended for those patients whose viral load is more than 10^6 GE per ml and the level of ALT remains increased constantly (by more than 2 times from the normal).

Figure 4. Dynamics of markers in acute viral hepatitis B

Hepatitis B virus (HBV) is a widely spread human infection caused by DNA-containing virus of hepatitis B belonging to the family Hepadnaviridae. Contamination with hepatitis B virus occurs at direct entry of the virus into blood (at parenteral interventions or hematotransfusions) or through mucous tunics, injuries of skin integuments (during sexual intercourse, vertical transfer, close household contacts).

The viral hepatitis B presents a serious problem for public healthcare due to its universal spread. At present in accordance with the WHO data the population infected with hepatitis B virus makes 500 million people. The total number of patients affected with chronic hepatitis B makes 50 million people. The incidence of HBV-infection in various regions varies in a wide range. The most unfavourable regions in this connection are the South-Eastern Asia and Africa where the incidence of the chronic infections reached 8-15 percent. In Russia the spread HBsAg is variable enough: in the European part of the country its incidence is limited to 1 percent, whereas in the Eastern Siberia it makes 4-5 percent and in republic of the Northern Caucasus, Yakutia and Tuva this figure comes up to as much as 8-10 percent.

Results of recently conducted researches play the leading role for confirmation of the diagnosis of viral hepatitis B, establishment of chronic viral hepatitis B, monitoring of activity of virus replication in bearers of HBsAg, monitoring of effectiveness of the antiviral therapy.

Serological diagnostics

Detection of HBsAg (superficial antigen) is extensively used at present as a screening test. HBcoreAg (HBeAg) is the major protein of nucleocapsid of the virus and is not secreted, that’s why it’s not detected in the blood serum. Antibodies to HBcoreAg, belonging to immunoglobulins of class M (anti-HBc IgM) and being a serological marker of the acute viral hepatitis B, develop at the height of the disease and might retain for 1 year. In a number of cases they might be the only marker of the acute viral hepatitis B. Antibodies belonging to immunoglobulins of class G (anti-HBc IgG), indicate current or previous infection.

HBeAg-antigen is a secreted protein. Its detection in the serum is an indirect sign of the active viral replication. HBeAg is detected in the blood serum of patients with acute viral hepatitis B at the height of the disease as well as in chronic forms concurring with the aggravation of the process. Serocconversion by HBeAg (appearance of antibodies to this antigen in the blood serum (anti-HBe)) is a manifestation of the favourable course of the disease that testifies to reduction of the virus replication activity.

The drawback of this group of methods is the impossibility to use them at low viral load, at contamination with mutant forms of the virus, at immunosuppression and for quantitative evaluation of HBV.
The reagent kits are adapted for automatic isolation of nucleic acids on the easyMAG unit (BioMerieux).

**Representative works. FRT format**

- Data by JOE/Yellow channel – detection of HBV DNA. Clinical samples are shown in red, control samples – in blue.

- Data by FAM/Green channel — detection of the internal control. Clinical samples are shown in red, control samples – in blue.

**Explanation:**

**Configuration of kits:**

- a “complete set reagent kit” includes reagents for extraction, amplification and detection;
- an “amplification reagent kit” (PCR-set) includes only amplification reagents.

**Kit types:**

- “Hot Start” is provided by a wax layer:
  - a kit is included in the complete set reagent kit (\(\square\));
  - a set includes ready to use PCR test tubes with the lower mixture applied under the wax;
  - a set includes vials with reagents not dispensed into PCR-test tubes.
- “Hot Start” is provided by a modified polymerase (TaqF) activated at heating:
  - a set includes vials with reagents not dispensed into PCR test tubes, a modified polymerase TaqF is used.

**Advantages of reagent kits**

- Use of the recombinant internal control sample allows control of all stages of analysis (RNA isolation, reverse transcription and PCR) and evaluation of PCR inhibitors effect on results of the test.
- Convenient and quick PCR format.

**Clinical material for examination**

<table>
<thead>
<tr>
<th>Type of clinical material</th>
<th>Recommended kits for extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma of peripheral blood</td>
<td>RIBO-sof= &amp; RIBO-Prep NucliSENS reagents for easyMAG</td>
</tr>
</tbody>
</table>

**FRT format – Fluorescence Detection in Real-Time Regime**

<table>
<thead>
<tr>
<th>Cat.No.</th>
<th>Name</th>
<th>Set</th>
<th>No. of tests</th>
<th>Type</th>
<th>Mark</th>
<th>Special equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR-V5 (RG,iQ)</td>
<td>AmpliSens® HBV-FRT</td>
<td></td>
<td>48</td>
<td>FAM/Green and JOE/HEX/Yellow</td>
<td></td>
<td>Rotor-Gene 6000 (Corbett Research), iCycler, iQ5 (BioRad)</td>
</tr>
<tr>
<td>R-V5 (RG,iQ)</td>
<td>AmpliSens® HBV-FRT</td>
<td></td>
<td>76</td>
<td>FAM/Green and JOE/HEX/Yellow</td>
<td></td>
<td>Rotor-Gene 6000 (Corbett Research), iCycler, iQ5 (BioRad)</td>
</tr>
<tr>
<td>R-V5-120 (RG,iQ,Mx)</td>
<td>AmpliSens® HBV-FL</td>
<td></td>
<td>120</td>
<td>FAM/Green and JOE/HEX/Yellow</td>
<td></td>
<td>easyMAG (BioMerieux), Rotor-Gene 6000 (Corbett Research), iCycler, iQ5 (BioRad), Mx3000P/Mx3005P (Stratagene)</td>
</tr>
</tbody>
</table>

**Advantages of format**

- Higher effectiveness of detection and lower labour intensity of detection.
- No risk of contamination.
- Reagent kits are adapted for a wide range of units for PCR in the real-time regime.
- Maximum test sensitivity.

**Analytical properties**

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>100 GE per ml, 20 GE per ml at use of the easyMAG unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>No cross reactions for adenoviruses (types 2, 3 and 7), cytomegalovirus, Epstein-Barr virus, chicken pox virus, hepatitis C virus, human immunodeficiency virus of type 1; human herpes virus, types 6 and 8; simplex herpes virus, types 1, 2; HPV types 6, 11, 16, 18, 33, 35</td>
</tr>
</tbody>
</table>

**Results of clinical tests**

Sensitivity and specificity of the test were evaluated in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute as compared to AmpliSens® HBV-Eph for detection of DNA of the hepatitis B virus by PCR method with electrophoretic detection of amplification products in agarous gel (FSP 42-0155-1135-01) for evaluation of the HCV viral load. The kit was tested on clinical samples (blood plasma) and HBV DNA standard samples of the company (SSC HBV DNA) - blood plasma containing HBV in different concentrations (10^4, 10^3 and 10^2 GE per ml). The experimental group included 20 patients with positive results by ELISA test for HBsAG or antibodies to hepatitis B virus and 20 samples of SSC HBV DNA. The total number of samples made 40. The control group was represented by samples of blood plasma of patients affected by other-etiology hepatitises and samples of healthy persons – donors. The total number of samples made 30. The tested kit was found more sensitive, which is accounted for its higher analytical sensitivity as compared to the AmpliSens® HBV-Eph reagent kit: SSC samples with concentration 100 GE per ml were detected only by the AmpliSens® HBV-FRT reagent kit. The rest of the samples produced complete concurrence of results obtained with the tested and reference kits. The specificity of the reagent kit made 100 percent.
Attention! The technology presents danger of contamination! A separate room and personnel are required for the detection!

The experimental group included 20 patients with positive results by ELISA test for HBsAG or antibodies to hepatitis B virus and 20 samples of SSC HBV DNA. The total number of samples made 40. The control group was represented by samples of blood plasma of patients affected by other-etiology hepatitis and samples of healthy persons — donors. The total number of samples made 30. The tested kit was found more sensitive, which is accounted for its higher analytical sensitivity as compared to AmpliSens® HBV-EPF reagent kit. The diagnostic sensitivity for the tested reagent kit AmpliSens® HBV-EPF made 100 percent, the specificity of the reagent kit made 100 percent.

Results of clinical tests
Sensitivity and specificity of the test were evaluated in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute as compared to AmpliSens® HBV-EPh reagent kit for detection of DNA of the hepatitis B virus by PCR method with electrophoretic detection of amplification products in agarous gel (FSP 42-0155-1135-01) for evaluation of the HCV viral load. The kit was tested on clinical samples (blood plasma) and HBV DNA standard samples of the company (SSC HBV DNA) - blood plasma containing HBV in different concentrations (10⁻⁴, 10⁻³ and 10⁻² GE per ml). The experimental group included 20

### Analytical properties

| Sensitivity | 500 GE per ml |
| Specificity | No cross reactions for adenoviruses (types 2, 3 and 7), cytomegalovirus, Epstein-Barr virus, chicken pox virus, hepatitis C virus, human immunodeficiency virus of type 1; human herpes virus, types 6 and 8; simplex herpes virus, types 1, 2, HPV types 6, 11, 16, 18, 33, 35. |

### Results of clinical tests

Sensitivity and specificity of the reagent kit were evaluated in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute as compared to the Avicenna-NVU-PCR test for detection of DNA of the hepatitis B virus by PCR method with electrophoretic detection of amplification products in agarous gel. The kit was tested on clinical samples (blood plasma). The experimental group included 51 patient with diagnosis of viral hepatitis B. The control group was represented by samples of blood plasma of patients affected by other-etiology hepatitises and samples of healthy persons – donors (57 samples). The total number of samples made 109. The diagnostic sensitivity for the tested reagent kit AmpliSens® HBV-EPh reagent kit made 97.9 percent, the specificity of the reagent kit made 100 percent.

### Analytical properties

| Sensitivity | 1000 GE per ml |
| Specificity | No cross reactions for adenoviruses (types 2, 3 and 7), cytomegalovirus, Epstein-Barr virus, chicken pox virus, hepatitis B virus, human immunodeficiency virus of type 1; human herpes virus, types 6 and 8; simplex herpes virus, types 1, 2, HPV types 6, 11, 16, 18, 33, 35. |
Reagent kits for detection of hepatitis B virus (HBV) DNA

The reagent kits are adapted for automatic isolation of nucleic acids on the easyMAG unit (BioMerieux).

Representative works. FRT format

Data by JOE/Yellow channel — detection of DNA HBV. Clinical samples are indicated in red, calibrators (standard samples) — in blue.

Data by FAM/Green channel — detection of DNA HBV. Clinical samples are indicated in red, calibrators (standard samples) — in blue.

Explanation:

Configuration of kits:

- a “complete set reagent kit” includes reagents for extraction, amplification and detection;
- an “amplification reagent kit” (PCR-set) includes only amplification reagents.

Kit types:

“Hot Start” is provided by a modified polymerase (TaqF) activated at heating:

- a kit is included in the complete set reagent kit

Advantages of reagent kits

- Use of the recombinant internal control sample allows control of all stages of analysis (RNA isolation and PCR) and evaluation of PCR inhibitors effect on results of the test.
- Simultaneous analysis of the tested sample and quantitatively characterized internal sample allows determination of HBV RNA concentration in the tested sample.
- Quantitative measurements in a wide range of concentrations.

Clinical material for examination

Type of clinical material | Recommended kits for extraction
---|---
Plasma of peripheral blood | RIBO-sorb → RIBO-Prep, NucliSENS reagents for easyMAG

FRT format – Fluorescence Detection in Real-Time Regime

<table>
<thead>
<tr>
<th>Cat.No.</th>
<th>Name</th>
<th>Set</th>
<th>No. of tests</th>
<th>Type</th>
<th>Mark</th>
<th>Special equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR-VS-M(RG,iQ,Mx)</td>
<td>AmpliSens® HBV-Монитор-FRT</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td>easyMAG (BioMerieux), iCycler, iQ5 (BioRad), Mx3000P/Mx3005P (Stratagene)</td>
</tr>
<tr>
<td>R-VS-M(RG,iQ,Mx)</td>
<td>AmpliSens® HBV-Монитор-FRT</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td>easyMAG (BioMerieux), iCycler, iQ5 (BioRad), Mx3000P/Mx3005P (Stratagene)</td>
</tr>
</tbody>
</table>

Analytical properties

| Sensitivity | 3 × 10^9 GE per ml Linear range of measurements: 3 × 10^9 — 10^8 GE per ml |
| Specificity | No cross reactions for adenoviruses (types 2, 3 and 7), cytomegalovirus, Epstein-Barr virus, chicken pox virus, hepatitis C virus, human immunodeficiency virus of type 1; human herpes virus, types 6 and 8; simplex herpes virus, types 1, 2; HPV types 6, 11, 16, 18, 33, 35. |

Results of clinical tests

Sensitivity and specificity of the test were evaluated in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute as compared to the Cobas Amplicor HBV Monitor test (Hoffmann La-Roche) for detection the viral load of HBV. The reagent kit was tested on clinical samples (blood plasma) and HBV HbsAg DNA standard samples (SSC 42-28-332-2000P), its dilutions with various concentrations as well as on HBV DNA standard samples of the company (SSC HBV DNA). The experimental group included 26 patients with positive results by ELISA for HbsAG (HbsAg Roche) or positive results for presence of DNA HBV in the kit AmpliSens® HBV-Eph (FSP 42-0155-1135-01), 12 samples of NRS standard and 36 SSC samples. The total number of samples made 71. The control group was represented by samples of blood plasma of donors with negative results of ELISA for presence of HbsAg antibodies or negative PCR results for presence of DNA HBV in the AmpliSens® HBV-Eph (FSP 42-0155-1135-01) kit — 12 samples. The difference between results (Δ lg), obtained on two reagent kits (the tested and reference ones) in the working range of the reference test-system didn’t exceed 0.21 lg, which testifies to comparability of results. The specificity of reagent kits was assessed on 12 blood plasma samples obtained from donors without antibodies to HBV. The specificity made 100 percent.
Hepatitis Delta Virus (HDV) and hepatitis G (HGV)

Hepatitis Delta Virus (HDV) and hepatitis G (HGV)

Hepatitis Delta Virus (HDV) is an RNA containing, hepatotropic viroid (uncompleted virus) belonging to Deltavirus family. HDV needs helper function of hepatitis B virus that provides to hepatitis delta virus (HDV) proteins of the superficial membrane (HBsAg). That's why HDV can replicate itself only in presence of HBV.

Ways of HDV-infection are analogous to transmission ways of hepatitis B (parenteral, sexual way, vertical mother-to-child transmission is also possible).

Endemic regions by HDV include central regions of the South America and Equatorial Africa. There are about 500 million people in the world who are infected with hepatitis B virus. Their HDV contamination in endemic regions might reach 60 percent.

HDV-infection might progress in two forms - coinfection and superinfection. Coinfection is the process of simultaneous contamination with viruses of hepatitis B and D. Superinfection is the process of contamination with hepatitis D virus of the patient with chronic hepatitis B or carrier of hepatitis B virus. Dynamics of viral markers in coinfec tion and superinfection is represented on Figures 5 and 6. Coinfection has features of acute grave infectious disease with a high risk of development of fulminant hepatitis (up to 20 percent). In superinfection when the infectious disease occurs in the changed tissue of the liver, in 70-80 percent of cases infection leads to quick development of cirrhosis.

Identification of HDV-infection on the background of hepatitis B is very important as the course of the disease, therapeutic approaches and forecast in hepatitis D are different from those in HGV.

Hepatitis G Virus

This is another virus causing post-transfusion hepatitis. The same as hepatitis C virus, hepatitis G virus belongs to the flavivirus group. The hepatitis G virus is detected with the help of PCR (serological methods are less reliable). It's detected in 1.5 percent of donors and in some patients with acute fulminant and chronic hepatitis. Hepatitis G might be characterized as “clinically quiescent infection”. Biochemical values of hepatitis G patients show for the most part affection of bile passages, which is a specific marker of hepatitis G. The coinfection of hepatitis G with hepatitises B, C or D is detected more frequently. The sources of the virus spread are patients with acute, chronic hepatitis G and carriers of HGV. The virus might be detected in serum, plasma, mononuclears of peripheral blood and saliva.

Modern laboratory diagnostics of HDV

HDV is studied insufficiently. The literature provides evidence on the fact that the immune response in HDV fails to comply with the virus replication cycle and a high frequency of HDV-infection is observed at this, that's why it's reasonable to use test systems based on PCR for diagnostics of HDV-infection. In addition to this, HDV RNA is the first diagnostic marker detected in the patient's blood, antibodies to D-Ag appear 2-3 weeks later. Detection of HDV RNA by PCR allows detection of the causative agent in the period of introduction of infection before seroconversion, which is very important for early diagnostics. Hepatitis D virus in coinfection as well as in superinfection suppresses replication of the hepatitis B virus. Lack of HDV DNA or low concentration of HBV DNA in blood plasma of the chronically infected patients in presence of cytolysis syndrome (or other manifestations of liver affection) is the indication to examination for HDV. It's necessary to take into account that suppression of HBV replication might be so expressed that it will lead to reduction of HBsAg level to undeterminable levels. That's why disappearance of HBsAg on the background of persisting signs of liver affection is also an indication for test for HDV.
Reagent kits for detection of hepatitis D (HDV) virus RNA in the clinical material

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### Clinical material for examination

<table>
<thead>
<tr>
<th>Type of clinical material</th>
<th>Recommended kits for extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical material for examination</td>
<td>RIBO-sorb® RIBO-Prep</td>
</tr>
</tbody>
</table>

- *a kit is included in the complete set reagent kit* (R)

### FRT format – Fluorescence Detection in Real-Time Regime (since Q4 Y2009)

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Name</th>
<th>Set</th>
<th>No. of tests</th>
<th>Type</th>
<th>Mark</th>
<th>Special equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>V3-F-FRT</td>
<td>AmpliSens® HDV-FRT</td>
<td>100</td>
<td>FAM/Green, JOE/Yellow</td>
<td>Rotor-Gene 6000 (Corbett Research)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Analytical properties

- **Sensitivity**: 500 GE per ml
- **Specificity**: No cross reactions for adenoviruses (types 2, 3 and 7), cytomegalovirus, Epstein-Barr virus, chicken pox virus, hepatitis B and C viruses, human immunodeficiency virus of type 1; human herpes virus, types 6 and 8; simplex herpes virus, types 1, 2; HPV types 6, 11, 16, 18, 33, 35.

### Results of clinical tests

The reagent kit was tested on clinical samples (blood samples). The experimental group included 150 patients with diagnosis virus hepatitises B+D. AmpliSens® HDV EPh reagent kit was used as a comparative system. The control group was represented by blood plasma of patients affected by other etiology hepatitises and samples of healthy persons – donors. The total number of samples made 30. The diagnostic specificity of the tested reagent kits made 100 percent, the diagnostic sensitivity – 100 percent.

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### FEP Format. End Point Fluorescence Detection

<table>
<thead>
<tr>
<th>Cat.No.</th>
<th>Name</th>
<th>Set</th>
<th>No. of tests</th>
<th>Type</th>
<th>Mark</th>
<th>Special equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>V3-F-FEP</td>
<td>AmpliSens® HDV-FEP</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td>Detectors ALA-1 (BioSan), Gene (DNA-Technology)</td>
</tr>
</tbody>
</table>

### Analytical properties

- **Sensitivity**: 500 GE per ml
- **Specificity**: No cross reactions for adenoviruses (types 2, 3 and 7), cytomegalovirus, Epstein-Barr virus, chicken pox virus, hepatitis B and C viruses, human immunodeficiency virus of type 1; human herpes virus, types 6 and 8; simplex herpes virus, types 1, 2; HPV types 6, 11, 16, 18, 33, 35.

### Results of clinical tests

The reagent kit was tested on clinical samples (blood samples). The experimental group included 150 patients with diagnosis virus hepatitises B+D. AmpliSens® HDV EPh reagent kit was used as a comparative system. The control group was represented by blood plasma of patients affected by other etiology hepatitises and samples of healthy persons – donors. The total number of samples made 30. The diagnostic specificity of the tested reagent kits made 100 percent, the diagnostic sensitivity – 100 percent.
Representative works. FEP format

1 - HDV-positive assay;
2-11 - HDV-negative assays;
12 - NCS (negative control sample);
13 - C- (negative control sample (PCR stage));
14 - C+ (positive control sample PCS cDNA HDV);
M - marker of molecular weight

Explanation:

Configuration of kits:
- a “complete set reagent kit” includes reagents for extraction, amplification and detection;
- an “amplification reagent kit” (PCR-set) includes only amplification reagents.
- a “reverse transcription and amplification reagent kit”

Kit types:
“Hot Start” is provided by a wax layer:
- a set includes ready to use PCR test tubes with the lower mixture applied under the wax
- a set includes vials with reagents not dispensed into PCR test tubes.
“Hot Start” is provided by a modified polymerase (TaqF) activated at heating:
- a set includes vials with reagents not dispensed into PCR test tubes, a modified polymerase TaqF is used.

Analytical properties

Sensitivity
5 x 10^3 GE per ml

Specificity
No cross reactions for adenoviruses (types 2, 3 and 7), cytomegalovirus, Epstein-Barr virus, chicken pox virus, hepatitis B and C viruses, human immunodeficiency virus of type 1; human herpes virus, types 6 and 8; simplex herpes virus, types 1, 2; HPV types 6, 11, 16, 18, 33, 35.

Reagent kits for detection of hepatitis G virus (HGV) RNA in clinical material

Clinical material for examination

<table>
<thead>
<tr>
<th>Type of clinical material</th>
<th>Recommended kits for extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma of peripheral blood</td>
<td>RIBO-sorb, RIBO-Prep</td>
</tr>
</tbody>
</table>

Results of clinical tests
The AmpliSens® HDV reagent kit was tested in clinical samples (blood samples). ELISA test system for detection of antibodies to HDV infection was used as a comparative system. The experimental group included 43 patients with diagnosis viral hepatitis D. The control group was represented by blood plasma of patients affected by other etiology hepatitises (105 samples) and samples of healthy persons – donors (35 samples) and bovine fetal serum samples (30 samples). The total number of samples made 170. The diagnostic specificity of the tested reagent kits made 97 percent, the diagnostic sensitivity – 97.6 percent.

Eph Format - Electrophoretic Detection

Attention! The technology presents danger of contamination! A separate room and personnel are required for the detection!

<table>
<thead>
<tr>
<th>Cat.No.</th>
<th>Name</th>
<th>Set</th>
<th>No. of tests</th>
<th>Type</th>
<th>Special equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV3-100-R0.5</td>
<td>AmpliSens® HDV-EPh</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TV3-100-R0.2</td>
<td>AmpliSens® HDV-EPh</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V3-200</td>
<td>AmpliSens® HDV-EPh</td>
<td>220</td>
<td></td>
<td></td>
<td>Electrophoretic chamber, gel-documentation system</td>
</tr>
<tr>
<td>V3-100-R0.5</td>
<td>AmpliSens® HDV-EPh</td>
<td>110</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V3-100-R0.2</td>
<td>AmpliSens® HDV-EPh</td>
<td>110</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analytical properties

Sensitivity
5 x 10^3 GE per ml

Specificity
No cross reactions for adenoviruses (types 2, 3 and 7), cytomegalovirus, Epstein-Barr virus, chicken pox virus, hepatitis B and C viruses, human immunodeficiency virus of type 1; human herpes virus, types 6 and 8; simplex herpes virus, types 1, 2; HPV types 6, 11, 16, 18, 33, 35.