AmpliSens® MultiPrime Real-Time PCR kit

HCV/HBV/HIV Testing of donated blood

- Simultaneous detection of HCV, HBV, HIV-1, and HIV-2
- Enhanced sensitivity compared to serological analysis
- Testing of both individual samples and mini-pools
- Possibility of usage in PCR workflow automation
- Compatibility with various nucleic acid extraction solutions
«AmpliSens® HCV/HBV/HIV-FL» Real-Time PCR kit

is intended for simultaneous detection of hepatitis C virus RNA (HCV), hepatitis B virus DNA (HBV), human immunodeficiency virus type 1 RNA (HIV-1) and type 2 RNA (HIV-2) in blood samples by Real-Time PCR.

«AmpliSens® HCV/HBV/HIV-FL» was developed by the Russian Central Research Institute for Epidemiology to provide safety control tests during blood transfusions, transplantations, and preparation of blood products. Investigation of plasma samples combined into mini-pools is also admissible.

- **Real-Time PCR** provides direct detection of viral RNA/DNA;
- **Plasma samples or their pools** are recommended for analysis. Serum, blood components, biopsy samples, etc. can also be used;
- **Extraction** is performed from large volume of clinical samples (up to 1 ml) to ensure maximal sensitivity of the test;
- **Analysis** is conducted in one tube, targets are independently detected in five or four channels (with or without HIV-2, correspondingly)

<table>
<thead>
<tr>
<th>HCV RNA</th>
<th>Green</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV DNA</td>
<td>Orange</td>
</tr>
<tr>
<td>HIV-1 RNA</td>
<td>Yellow</td>
</tr>
<tr>
<td>HIV-1 RNA</td>
<td>Crimson</td>
</tr>
<tr>
<td>Exogenous internal control</td>
<td>Red</td>
</tr>
</tbody>
</table>

Testing of blood - two approaches:
- **consequent:**
  - PCR analysis is performed only for samples determined as negative in ELISA.
  - cost-effective
  - time-consuming
- **parallel:**
  - PCR and ELISA are performed for all plasma samples.
  - comprehensive
  - expensive

Options for automation

Soft-hardware complex for testing of donated blood

- Pooling
  - Neon 100-1-8 (XIRIL)

- Nucleic acid extraction and preparation for PCR analysis
  - QIASymphony SP/AS (QIAGEN)

- Amplification and detection
  - Rotor-Gene Q (QIAGEN)

Special software for total process control and analysis of results.

www.pcrdiagnostics.eu
Plasma Pooling

«AmpliSens HCV/HBV/HIV®-FL» reagent kit can be used for analysis of individual samples as well as for several plasma samples combined into a mini-pool. Plasma pooling allows reducing price of analysis and increasing laboratory throughput.

Number of plasma samples recommended for mini-pools is 4-10 with total volume of 1000 μl.

**Plasma pooling techniques:**

**Single pooling** – every single sample is a part of only one pool.
100 samples = 10 pools

This way of pooling is recommended if:
- frequency of positive samples in PCR analysis is less than 1% (applicable if samples are negative in ELISA);
- there is no need to decode mini-pools promptly.

**Double pooling (matrix pooling)** – every single sample is a part of 2 pools.
100 samples = 20 pools

This way of pooling is recommended if:
- frequency of positive results after PCR analysis is ≥1% (PCR analysis is done in parallel to ELISA);
- there is a necessity in fast decoding of positive mini-pools.

Nucleic Acid Extraction

**Methods recommended for nucleic acid extraction from blood plasma**

**Manual methods:**
- RIBO-prep – precipitation method (from 100 μl);
- RIBO-sorb – sorbent method (from 100 μl);
- MAGNO-sorb – magnetic bead-based method (from 200 or 1000 μl).

**Semi-automatic methods:**
- «NucliSENS® easyMAG®» workstation («bioMérieux», France) with use of reagent kit EM-plus (from 100-1000 μl).

**Automatic methods:**
- Neon 100-1-4 workstation (Xiril, Switzerland) for pooling and QIAsymphony SP/AS (QIAGEN, Germany) for nucleic acid extraction and sample preparation for PCR.

**Sensitivity**

<table>
<thead>
<tr>
<th>Extraction volume, μl</th>
<th>Extraction method</th>
<th>Analytical sensitivity (detection in a pool*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HCV, IU/ml</td>
</tr>
<tr>
<td>100</td>
<td>RIBO-prep RIBO-sorb «NucliSENS® easyMAG® » + EM-plus</td>
<td>100</td>
</tr>
<tr>
<td>200</td>
<td>MAGNO-sorb</td>
<td>50</td>
</tr>
<tr>
<td>1000</td>
<td>MAGNO-sorb «NucliSENS® easyMAG® » + EM plus QIAsymphony Virus/Bacteria Midi Kit</td>
<td>10</td>
</tr>
</tbody>
</table>

*Sensitivity for individual samples is multiply dependent on dilution during pooling

Advantages of «AmpliSens® HCV/HBV/HIV-FL» Real-Time PCR kit

- Multiplex reaction in one tube saves time and costs
- Analytical sensitivity and specificity correspond to the highest worldwide standards
- Mini pools (up to 10 samples in each pool) can be tested along with individual samples
- Combination of reverse transcription and amplification reactions simplifies the protocol and reduces the number of possible mistakes
- Automatic or manual extraction from 1000 μl of blood plasma ensures maximal sensitivity
- Test reveals infected persons even if ELISA results are negative and serve as confirming investigation for ELISA-positive samples
- Protected RNA extraction controls and DNA amplification controls allow monitoring of every step of analysis
- Automated soft-hardware complex was developed to analyze samples and interpret results
- Real-Time PCR kit was approved in state trials and evaluated at large blood transfusion stations and transplantation centers

Ordering Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR-V62-Ms (RG, Dt)-CE</td>
<td>«AmpliSens® HCV/HBV/HIV-FL»</td>
<td>Detection of Hepatitis C (HCV) RNA, B (HBV) DNA, HIV-1 RNA, and HIV-2 RNA (5 optical channels are required). Complete set of kits (nucleic acid extraction kit «MAGNO-sorb», reverse transcription and Real-Time PCR kits) for use with RG and Dt. <strong>100 tests</strong></td>
</tr>
<tr>
<td>R-V62(RG, Dt)-CE</td>
<td>«AmpliSens® HCV/HBV/HIV-FL»</td>
<td>Detection of Hepatitis C (HCV) RNA, B (HBV) DNA, HIV-1 RNA, and HIV-2 RNA (5 optical channels are required). Reverse transcription and Real-Time PCR kits for use with RG and Dt. Kits are recommended for RNA/DNA extraction: MAGNO-sorb variant 1000, reagents for «NucliSENS® easyMAG» (+ reagent set EM-plus), RIBO-prep, RIBO-sorb. <strong>100 tests</strong></td>
</tr>
<tr>
<td>R-V50-4x (RG, IQ, Mx, Dt)-CE</td>
<td>«AmpliSens® HCV/HBV/HIV-FL»</td>
<td>Detection of Hepatitis C (HCV) RNA, B (HBV) DNA, and HIV-1 RNA (4 optical channels are required). Reverse transcription and Real-Time PCR kits for use with RG, IQ, Mx, and Dt. Kits are recommended for RNA/DNA extraction: MAGNO-sorb variant 1000, reagents for «NucliSENS® easyMAG» (+ reagent set EM-plus), RIBO-prep, RIBO-sorb. <strong>100 tests</strong></td>
</tr>
<tr>
<td>K2-16-1000-CE</td>
<td>MAGNO-sorb</td>
<td>Kit for DNA/RNA extraction from 1000 μl of human plasma and blood serum. Magnetic bead-based method. For manual or automated extraction using Neon 100-1-8 (Xiril’s Robotic Workstation). <strong>100 tests</strong></td>
</tr>
<tr>
<td>K2-16-200-CE</td>
<td>MAGNO-sorb</td>
<td>Kit for DNA/RNA extraction from 200 μl of human plasma and blood serum. Magnetic bead-based method. For manual or automated extraction using Neon 100-1-8 (Xiril’s Robotic Workstation). <strong>100 tests</strong></td>
</tr>
<tr>
<td>K2-9-Et-100-CE</td>
<td>RIBO-prep</td>
<td>Kit for RNA/DNA extraction from blood plasma, liquor, saliva, amniotic fluid, and swabs. Precipitation method. <strong>100 tests</strong></td>
</tr>
<tr>
<td>K2-1-Et-100-CE</td>
<td>RIBO-sorb</td>
<td>Kit for RNA/DNA extraction, universal. Silica sorption method. <strong>100 tests</strong></td>
</tr>
<tr>
<td>K2-15-96-CE</td>
<td>EM-plus</td>
<td>Additional reagent kit for RNA/DNA extraction on automatic stations «NucliSENS® easyMAG» with use of «AmpliSens® HCV/HBV/HIV-FL». <strong>100 tests</strong></td>
</tr>
</tbody>
</table>