Article number and Product Name
P0042 HCV-RNA quantification panel.

Intended Use
The HCV-RNA quantification panel provides a consistent standard across NAT methods, enabling blood screening laboratories and diagnostic manufacturers to assess the linearity and accuracy of quantitative molecular diagnostic test procedures for the detection of Hepatitis C virus (HCV) in blood samples. This product can be used with (real time) amplification and hybridisation methods. It also can be used as a calibration panel in quantification of secondary HCV-RNA standards. This product is not for diagnostic use. The panel should not be used for the evaluation of HCV-Ag assays.

Summary and Explanation
Testing Hepatitis C viral load is imperative to monitor success of patient treatment and clinical research on Hepatitis C. The quantification panel provide standardised samples with a known viral load which can be used to compare, adjust assays or internal, secondary laboratory standards. The panel consist of a standard dilution series in negative human plasma. The concentrations of HCV-RNA quantification panel members are primarily expressed in bDNA copies/ml and secondarily in IU/ml. The HCV standard was diluted in a pool of plasma units that tested negative for viral markers in individual donation NAT and serology testing. The viral concentrations in the plasma pool are ensured by gravimetrically recorded dilutions from calibrated viral stock solutions stored at −70°C.

Historical calibration against EUROHEP and 1st WHO standard for HCV-RNA (genotype 1)
Primarily quantification is done by multiple testing (n=27) in the Siemens Versant bDNA3.01 assay (C.I. +/- 3 %). The quantification is confirmed by limiting dilution analysis using nucleic acid blood screening assays (unpublished results). The HCV-RNA genotype 1 standard has been calibrated against the EUROHEP HCV-RNA genotype 1 standard in a proficiency study3,4,5 and the first WHO HCV-RNA standard6,7. The concentrations given for each samples are obtained by gravimetrically controlled diluting of the HCV-RNA genotype 1 standard.

Principles of the Evaluation Procedure
HCV-RNA quantification panel members have been carefully formulated to mimic human plasma specimens containing relevant concentrations of HCV-RNA. Accuracy and linearity can be evaluated by plotting the given log (concentration) against the log (measured concentration). The linearity is evaluated by calculating the correlation coefficient for different concentration ranges. The slope of the line can be used to relate the measured concentration to the given concentration in copies/ml, IU/ml. The accuracy is estimated for each sample by applying descriptive statistics on log (measured concentrations).

HCV-RNA quantification panel reagents

<table>
<thead>
<tr>
<th>Panel member</th>
<th>HCV-RNA concentration in copies/ml and in IU/ml (between brackets)</th>
<th>Quantity (ml per vial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$2.5 \times 10^5$ ($1.2 \times 10^5$)</td>
<td>1 x 1.0 ml</td>
</tr>
<tr>
<td>2</td>
<td>$5.0 \times 10^4$ ($2.3 \times 10^4$)</td>
<td>1 x 1.0 ml</td>
</tr>
<tr>
<td>3</td>
<td>$2.5 \times 10^3$ ($1.2 \times 10^3$)</td>
<td>1 x 1.0 ml</td>
</tr>
<tr>
<td>4</td>
<td>$5.0 \times 10^2$ ($2.3 \times 10^2$)</td>
<td>1 x 1.0 ml</td>
</tr>
<tr>
<td>5</td>
<td>$5.0 \times 10^1$ ($1.2 \times 10^1$)</td>
<td>1 x 1.0 ml</td>
</tr>
</tbody>
</table>

Precautions
Warning: The HCV-RNA quantification panel members contain infectious HCV particles, which are not manipulated or inactivated, and are potentially biohazardous. Observe the universal precautions for prevention of transmission of infectious agents when handling these materials8,9. Although the normal human plasma used in the production of this panel was negative for infectious disease markers the quantification panel members should be handled as if capable of transmitting (unknown) infectious agents. Do not pipette by mouth. Use personal protective equipment, including lab coats, gloves and safety glasses. Do not eat, drink or smoke in areas where the members are handled. Disinfect liquids, materials or spills with a 0.5% sodium hypochlorite solution or equivalent. Dispose of all materials and liquids used in procedure as if they contained pathogenic agents.
Storage Instructions
It is necessary to store the panel at –70°C or lower to ensure no degradation can occur and the given quantification is correct. Discard any unused material after the first use. Any panel members that appear cloudy or contain precipitates after thawing should be discarded.

Instructions for Use
Thaw the panel members quickly in a water bath at 37°C to avoid formation of cryo-precipitates, mix gently during thawing until ice clot just has disappeared. Immediately after thawing, vortex briefly, and give a short spin before releasing screw cap from the vials. The panel members are now ready use.

The panel members should be handled and tested in a manner identical to that required for clinical specimens run in the test procedure being evaluated. Follow the manufacturers or testing laboratory instructions and recommendations for the handling and testing of clinical specimens.

After thawing the samples should be used within 24 hours, for a short period you can store at 2-8°C. In case you want to aliquot members for multiple use samples must be snap frozen using liquid nitrogen.

Limitations
HCV-RNA quantification panel members are not intended to replace the internal calibrators integral to in vitro diagnostic (IVD) test kits, but may be used as external, independent standardised samples for assessment on quantification and linearity of nucleic acid assays.

Key to Symbols Used

<table>
<thead>
<tr>
<th>Date of manufacturing</th>
<th>Manufacturer</th>
<th>Lot number</th>
<th>Catalogue number</th>
<th>Store at -70°C or lower</th>
<th>Biological substance Category B</th>
</tr>
</thead>
</table>

Literature

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