P0042 HCV-RNA
(500 to 250,000 copies/ml)

RUO

REF P0042

The kit insert contains a detailed protocol and should be read carefully before testing the run control to ensure optimal performance

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Intended Use

The P0042 HCV-RNA (500 to 250,000 copies/ml) 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) RNA in blood samples. This product can be used with amplification and hybridisation methods and is useful for testing the linearity and accuracy of quantitative NAT methods as well as for acceptance of new diagnostic reagent lots, NAT system validation and training. It also can be used as a calibration panel in quantification of HCV-RNA concentrations. This product is for research use only.

Key to Symbols Used

Manufacturer

Lot number

Catalogue number

Research use only

Country of origin

Store below -65°C

Expiry date

Contains human blood derivatives

Caution

Biological substance Category B

Contents

Read instructions for use

Summary and Explanation

The P0042 HCV-RNA (500 to 250,000 copies/ml) panel is designed for testing the accuracy of quantification by NAT methods. The reference panel helps ensure that NAT procedures for HCV-RNA are properly validated, and that test results are consistent across manufacturers, testing laboratories, operators, platforms and assay formats. The P0042 HCV-RNA (500 to 250,000 copies/ml) panel has been prepared from a well characterised HCV-RNA genotype 1 plasma standard. Dilutions of this S0009 HCV-RNA genotype 1 standard have been tested in multiple replicates against the EUROHEP and WHO HCV genotype 1 plasma standards in international proficiency studies and WHO collaborative studies[1-5]. The test results obtained over time with the Bayer Versant bDNA 3.0 assay[6,7] were used to calibrate the S0009 HCV-RNA genotype 1 standard in copies/mL. This assay was used as reference measurement method because the purified in-vitro RNA transcripts were quantified in RNA molecules by three analytical chemical methods[6]. The calibration of the S0009 HCV-RNA genotype 1 standard in International Units(IU)/mL was performed in the bDNA 3.0 assay and confirmed by probit analysis on data from different analytical sensitivity studies[8-12]. A conversion factor of 2.73 (1.4 – 4.8) copies per IU was established based comparison with the 1st WHO 96/970 and 2nd WHO 96/978 HCV genotype 1 standards. It must be emphasized that this conversion factor does not hold for the later WHO replacement standards. The traceability of the IU values assigned to consecutive WHO standards was lost due to stability issues and method dependency of the calibration results in WHO collaborative studies. The HCV-RNA concentrations given Table 1 are based on gravimetrically controlled dilutions of the S0009 HCV-RNA genotype 1 standard. The S0009 HCV standard was diluted in a pool of plasma units that tested negative for blood borne pathogens in molecular and serologic blood screening assays. The S0009

HCV-RNA genotype 1 standards and reference panels prepared from this standard have found to be stable for more than two decades when stored below –65°C.

Kit contents (materials provided)

Five (5) panel members; polypropylene tubes (5 mL) with screw caps, each containing 1.0 mL. The composition is given in table 1

Table 1. Composition of P0042 HCV-RNA (500 to 250,000 copies/mL) panel.

Panel member	HCV-RNA concentration in copies/mL (95% CI)	HCV-RNA concentration in IU/mL (95% CI)	Quantity (mL per vial) + overfill
1	2.50 (2.43 - 2.57 x 10^5	9.2 (5.2 - 17.9) x 10^4	1 x 1.0 mL
2	5.00 (4.87 - 5.15) x 10^4	1.8 (1.0 - 3.6) x 10^4	1 x 1.0 mL
3	2.50 (2.43 - 2.57) x 10^4	9.2 (5.2 – 17.9) x 10^3	1 x 1.0 mL
4	5.00 (4.87 - 5.15) x 10^3	1.8 (1.0 – 3.6) x 10^3	1 x 1.0 mL
5	5.00 (4.87 - 5.15) x 10^2	1.8 (1.0 – 3.6) x 10^2	1 x 1.0 mL

Materials required but not supplied

The test kits and liquid handling devices provided by the NAT manufacturer, a water bath of 37°C for quickly thawing of run control and a vortex instrument for thorough mixing of samples are not provided.

Storage instructions

P0042 HCV-RNA (500 to 250,000 copies/mL) panel should be stored at or below –65°C for a maximum of ten years after preparation of the panel batch and not be used after the expiry date. Once thawed the samples should be used immediately on the test platform. Do not refreeze the samples after thawing to prevent formation of cryoprecipitates. Any sample that appears cloudy or contains precipitates after thawing and mixing should be discarded.

Warning and precautions

P0042 HCV-RNA (500 to 250,000 copies/ml) panel members contain infectious hepatitis C virus particles and are potentially infectious. The matrix is prepared from human blood plasma that tested negative for blood borne viruses (HBV-DNA, HCV-RNA, HIV-RNA, HBsAg, anti-HBc, anti-HIV, anti-HCV and anti-*Treponema pallidum*). No test method can offer complete assurance that products derived from human blood cannot transmit (unknown) infectious agents. Observe the universal precautions for prevention of transmission of infectious agents when handling these materials[13,14].

- 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 run control is handled.
- Disinfect spills using a 0.5% hypochlorite solution (1:10 v/v household bleach) or equivalent disinfectant.
- Dispose unused or spilled materials according to the normal practices for biological waste disposal in your institution.
- If precipitates are visible, mix the samples for 2 minutes thoroughly.
- Once thawed, do not re-freeze and thaw the samples to avoid formation of cryoprecipitates that could alter reactivity or cause pipetting errors in the automated sampling systems.

- Store panel members in an upright position.

Reagent preparation

- Thaw panel members quickly in a water bath at 37°C.
- Mix gently during thawing until contents are just thawed.
- Immediately after the ice clot has disappeared remove the tube from the water bath.
- Before testing allow the test tube to adapt to room temperature.
- Mix the samples thoroughly prior to use with a vortex instrument.
- Give a short spin in a centrifuge to remove liquid before releasing screw cap from vial.
- Minimise the time from thawing until testing of the control samples.
- Test run control sample immediately after thawing.

Interpretation of Results

Precision

Real time HCV-RNA Polymerase Chain Reaction (PCR) or Transcription Mediated Amplification (TMA) assays report Ct values and/or quantitative HCV-RNA values expressed in either copies/mL or IU/mL. The dilution factors between the subsequent panel members is exact (less than 0.5% variation as gravimetrically recorded). Consequently, the distance in Ct value between panel members should be $\log_2$ (10) for the 10-fold dilutions and 1.00 for 2-fold dilutions. For calculating the precision of quantitative results in copies/mL or IU/mL a log transformation is required. On the log transformed values one can calculate the 95% confidence interval (95% CI) assuming a normal distribution.

Accuracy

The panel members are quantified in copies/ml based on the previously available bDNA 3.0 assay and in IU/mL values assigned to the 1st and 2nd WHO 96/970 and 96/978 standards (which were lyophilised batches of the same source material). It must be emphasized that current quantitative HCV-NAT methods may be calibrated on WHO replacement standards which are no longer traceable to the 1st and 2nd WHO standards used for calibration of the P0042 HCV-RNA reference panel. Even if historic data were analysed at the time only the 1st and/or 2nd WHO standards were available different quantitative values were reported on S0009 HCV-RNA genotype standard dilutions (unpublished data, Table 2). The accuracy (95% CI) in Table 2 was calculated on log transformed values assuming a normal distribution.

Table 2. Quantification of the S0009 HCV-RNA genotype 1 standard by different methods.

Assay	n	Accuracy (95% CI)
Roche Amplicor 1.0	90	64 (59 – 70) %
Roche COBAS Amplicor 2.0	73	70 (65 – 74) %
Roche MWP Amplicor 2.0	35	63 (56 – 71) %
Bayer bDNA 2.0	43	156 (149 – 187) %
Siemens bDNA 3.0	27	100 (97 – 103) %

Limitations

- P0042 HCV-RNA (500 to 250,000 copies/ml) must not be substituted for the mandatory controls or calibrators provided with NAT test kits for calculating the cut-off, quantitative values and/or criteria for releasing test results.

- The assigned quantitative values to the P0042 panel members in IU/ml are traceable to the 1st and 2nd WHO 96/970 and 96/978 HCV-RNA genotype 1 standards and are not traceable to the IU/mL values assigned to the later 3rd, 4th and 5th WHO replacement standards. Moreover, calibration results of different HCV-RNA standards were found to be method dependent. Therefore, the P0042 HCV-RNA (500 to 250,000 copies/ml) panel should not be used for establishing accuracy of quantitative NAT results expressed in IU/mL.

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Biologicals Quality Control B.V.
De Droogmakerij 31h
1851 LX Heiloo
The Netherlands

Tel: +31 (0)72 2020 730
Internet: www.bioQControl.com

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