Bio QControl

DRAFT

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) genotype 1 in blood samples. This product can be used with amplification and hybridisation methods and is useful for testing the linearity and accuracy, qualification of new diagnostic kit lots or 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.

Summary and Explanation

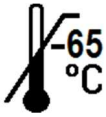
The P0042 HCV-RNA (500 to 250,000 copies/ml) panel is designed for testing the analytical sensitivity or quantification limits of NAT methods. The reference panel helps ensure that NAT procedures for HCV-RNA are properly validated, and that test results are consistent across manufacturers, testing laboratories, operators, platforms and assay formats. The P0042 HCV-RNA (500 to 250,000 copies/ml) has been prepared from a well characterised HCV-RNA genotype 1 plasma standard. Multiple testing in dilutions of the different HCV-RNA plasma standards in the Bayer Versant bDNA 3.0 assay[1,2] enabled us to calibrate the HCV-RNA standard. The quantification is confirmed by limiting dilution analysis using nucleic acid blood screening assays (unpublished results).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 proficiency studies[3,4,5] and the first WHO HCV-RNA standard[6,7]. The concentrations given for each samples are obtained by gravimetrically controlled diluting of the HCV-RNA genotype 1 standard. Calibration in other assays may yield a slightly different conversion factor caused by primer/probe matching as well as the effect of lyophilisation on extraction efficiency for the assigned value in IU/ml. The HCV-RNA-genotype 1 standard is a plasma pool prepared from interdicted donations. 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 ≥–65°C.

Key to Symbols Used

Manufacturer

Lot number

Catalogue number



Store below -65°C

Research use only

Expiry date

Contents

Caution

Read instructions for use

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 the panel.

Panel member	HCV-RNA concentration copies/ml (95 % Confidence interval)	IU/ml (calibrated against 1st International WHO standard ref. 96/790)	Quantity (ml per vial)
1	$2.50\ (2.43\text{-}2.57).10^5$	$9.16.10^4$	1 x 1.0 ml
2	$5.00\ (4.87\text{-}5.15).10^4$	$1.83.10^4$	1 x 1.0 ml
3	$2.50\ (2.43\text{-}2.57).10^4$	$9.16.10^3$	1 x 1.0 ml
4	$5.00\ (4.87\text{-}5.15).10^3$	$1.83.10^3$	1 x 1.0 ml
5	$5.00\ (4.87\text{-}5.15).10^2$	$1.83.10^2$	1 x 1.0 ml

Materials not provided

Pipettes or pipetting devices for use in IVD test systems.

Storage instructions

The run controls should be stored at or below -65°C. Once thawed the panel members should be used within 8 hours. During this period, when not in use, store sample at 2-8°C[18]. Do not refreeze the panel member after thawing to prevent formation of cryoprecipitates. Any panel member 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) contain infectious Hepatitis C virus particles and is infectious to humans. 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[8,9].

- 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 controls 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 panel members for 2 minutes thoroughly.
- Once thawed, do not re-freeze and thaw the panel members 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 the panel members quickly in a water bath at 37°C.
- Mix gently during thawing until contents are just thawed.
- Immediately after thawing remove the panel member tubes from the water bath.

- Vortex the run control.
- Give a short spin in a centrifuge to remove liquid before releasing screw cap from vial.
- Minimise the time period from thawing until usage of the panel members.
- Use within 8 hours after thawing
- After thawing when not in use: store at 2-8°C

Interpretation of Results

Precision

(Real time PCR) quantitative HCV-RNA tests report Ct values and/or [HCV-RNA] in either copies/ml or IU/ml. The dilution factor 10 between the subsequent panel member is exact (less than 0.5% variation as gravimetrically recorded). As a consequence the distance in Ct value between panel members should be $^{2}\log(10)$ for the 10-fold dilutions and 1.00 for the 2-fold dilutions. For other quantitative results please apply a log transformation. On the log transformed results one can calculate precision assuming a normal distribution.

Accuracy

The panel members are quantified in copies/ml using bDNA 3.0 and IU/ml. The quantification in IU/ml originate from the WHO studies[6,7] . We used the assigned quantification, the WHO does not define confidence intervals. The accuracy in other assays in given in the table 2. You can use the log transformed concentrations for calculations assuming a normal distribution.

Table 2 quantification of the HCV-RNA genotype 1 standard in other assays.

Assay	n	accuracy	95% Confidence interval	
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, concentrations and/or criteria for releasing test results.
- The assigned quantification in IU/ml relates to the 1st WHO HCV-RNA standard, we did not calibrate on the next generations WHO standards. Therefore P0042 HCV-RNA (500 to 250,000 copies/ml) should not be used for establishing accuracy of quantitative NAT results expressed in IU/mL. For this purpose only dilutions of the current WHO International Standard can be used.
- The panel is for research use only

References

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