Bio QControl

HEV-RNA reference panels

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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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Overview HEV-RNA reference panels

This insert describes the hepatitis E virus (HEV)-RNA standard dilution panels which can be used to establish the analytical sensitivity of NAT assays and determination of the quantification limit of quantitative NAT assays. Table 1 presents an overview of the available HEV-RNA reference panels for this purpose.

Table 1 HEV-RNA reference panels

Catalogue nr.	Product name	number samples
P0274	P0274 HEV-RNA genotype 3a	8
P0262	P0262 WHO HEV-RNA#	6

customized product

Intended Use

The HEV-RNA reference panels provide a consistent standard across NAT methods, enabling laboratories to assess the analytical sensitivity and quantification limits of molecular diagnostic test procedures for the qualitative and quantitative detection of HEV-RNA in plasma samples. This product can be used with amplification methods, including transcription mediated amplification (TMA) and real-time polymerase chain reaction (PCR) assays. The HEV-RNA reference panels are useful for establishing the lower limit of detection (LOD), the lower limit of quantification (LOQ), NAT reagent batch acceptance, NAT system validation and training. The panel members above the LOQ could also be used to determine the accuracy and precision of quantitative NAT methods. The product is for research use only and not for diagnostic use.

Key to Symbols Used

Manufacturer

Lot number

Catalogue number

Store below -65°C

Research use only

Biological substance category B

Date of manufacturing

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Summary and explanation

The HEV-RNA reference panels help ensure that NAT assays for HEV-RNA are properly validated, and that test results are consistent across manufacturers, testing laboratories, operators, platforms and assay formats. The secondary standard dilution panel is prepared from a plasma standard calibrated against the WHO 6329/10 standard for HEV-RNA[1,2]. For preparation of the reference panel, the HEV-RNA standard is diluted in a pool of plasma units that tested negative for viral markers in NAT and serology tests. Lot-to-lot consistency of the viral concentrations in the reference panels is ensured during manufacturing by gravimetrically recorded dilutions from calibrated viral stock solutions, stored at ≤ –65°C.

Traceability of HEV-RNA concentration in standard

Traceability and securing continuous and stable performance of HEV-RNA reference panels is based on the WHO 6329/10 standard[1,2] that was used for calibration of a secondary HEV-RNA standard. This is a plasma unit interdicted by the HEV-RNA screening program of the Sanquin Blood Supply Foundation (Amsterdam, the Netherlands)[3-5]. The concentration in the plasma unit was measured by replicate real time PCR tests against the WHO 6329/10 standard and was reported to be 54,000 IU/mL.

In the WHO collaborative study[1] the quantitative assays reported 1.33 copies per 1.00 IU and the qualitative assays 1.18 copies per 1.00 IU (after exclusion of laboratories with outlier test results). The geometrical mean value was 1.25 copies per 1.00 IU. When comparing the 50% LODs on the WHO PEI 6329/10 genotype 3a standard and *in vitro* HEV RNA genotype 3 transcripts in the package insert of the Procleix HEV assay[6], a conversion factor of 1.60 copies per 1.00 IU could be estimated.

Further calibration experiments are required to more accurately quantify the secondary HEV standard in IU/mL and cp/mL. The consistency in manufacturing is guaranteed by the gravimetrical records of the dilution steps from 54,000 IU/mL to 300 IU/mL.

Materials Provided

Table 2 presents the HEV-RNA concentration of the panel members in the HEV-RNA reference panels. The samples are filled off in polypropylene tubes (10 mL) with screw caps, containing 4.0 mL. (Samples of customized product P0262 were aliquoted in 5.0 mL volumes). For the secondary standard dilution panel P0274 a confidence interval of the HEV-RNA concentration in IU/mL is given based on the available calibration measurements against the WHO standard.

Table 2. Composition of HEV-RNA standard dilution panels

Reference panel	Member-ID	IU/mL (95% CI)#
P0274 HEV-RNA genotype 3a	B4266-xxx-01	300 (230-391)
	B4266-xxx-02	100 (77-130)
	B4266-xxx-03	30 (23-39)
	B4266-xxx-04	10 (7.7-13)
	B4266-xxx-05	3 (2.3-3.9)
	B4266-xxx-06	1 (0.77-1.30)
	B4266-xxx-07	0.3 (0.23-0.39)
	B4266-xxx-08	0.1 (0.08-0.13)
P0262 WHO HEV-RNA 6329/10	B4260-xxx-01	90
	B4260-xxx-02	30
	B4260-xxx-03	10
	B4260-xxx-04	3
	B4260-xxx-05	1
	B4260-xxx-06	negative

the quantification of the WHO standard in panel P0262 is absolute, no confidence interval is present.
The tube identification is a Byyyy-xxx-number, where yyyy is product specific and xxx the sequential batch number. The identification is present in the bar-code and explained on the tube label.

Materials not provided

Test kit and pipettes or pipetting devices for use in IVD test systems.

Storage Instructions

It is recommended that the panel is stored at –65°C or lower to ensure highest quality. At this temperature the panel is stable. Discard any unused material after the first use. Any panel members that appear cloudy or contain precipitates after thawing should be discarded.

Warning and precautions

Warning: The HEV-RNA reference panel members contain infectious HEV and are potentially bio-hazardous[7,8]. Apply the universal precautions for prevention of transmission of infectious agents when handling these materials[9,10]. Although the normal human plasma used in the production of this panel was negative for blood borne infectious disease markers the reference panel members should be handled as if capable of transmitting (unknown) infectious agents.

- **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 tube from the water bath.
- Mix the panel member(s).
- Give a short spin in a centrifuge before releasing screw cap from vial.
- Minimise the time period from thawing until usage of the panel members.
- The panel member should be handled and tested in a manner identical to that of clinical specimens in the test procedure being evaluated.
- **Do not refreeze panel members after thawing. When the panel is tested multiple times organize this within 8 hours after thawing. When not placed in the robot store samples at 2-8°C.**

Interpretation of Results

Lower Limit of detection (LOD)
For establishing the detection limit of NAT assays is the whole panel needs to be tested multiple times. It is recommended to test the concentrations with intermediate (between 20% and 100%) reactivity at least 12, and preferably 24 times. The proportions of reactive results are usually interpreted by probit analysis[11] for which results from 100% until below 50% reactivity rate should be included. At least two concentrations with intermediate reactivity should be available. Apply log transformation on the concentrations before calculating the LODs by probit analysis. It is recommended to report both the 50% and 95% LOD for interpretation of the analytical sensitivity . The limit of detection is often defined as the 95% LOD with the 95% confidence interval (CI).
Lower Limit of Quantification (LOQ).
The quantitation limit of an individual analytical procedure is the lowest amount in a sample which can be quantitatively determined with sufficient precision and accuracy.
Checking amplification efficiency.
For quantitative NAT assays the relation between ^{2}log(concentration) and ^{2}log(quantitative results) or Ct value can be judged using linear regression. Ideally the slope of the curve should be -1.00. If the result is different consider to remove lower concentrations with intermittent reactivity. The slope is accepted when the confidence interval of the slope overlaps -1.00
Calculation of precision.
The precision of quantitative NAT results becomes less with lower concentrations near the LOQ. This can be examined by the coefficient of variation (%CV) of replicate tests on a panel member. The %CV is the ratio of the standard deviation (SD) to the mean.

Calculation of accuracy

The accuracy of a quantitative result is represented by the distance of the measured value to the value assigned to the panel member. Calculate the mean and SD of the replicate tests of each standard dilution above the LOQ. Use the highest concentrations with the lowest %CV for calculating delta = Log(concentration assigned) minus log(concentration measured) for each measurement. The accuracy = $10^{-\text{average delta}}$

Excel spreadsheets for performing the calculations can be made available on request.

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