P0041 HBV-DNA genotype A Quant (10 – 10,000,000 copies/mL)

RUO

REF P0041

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

P0041 HBV-DNA genotype A Quant (10 to 10,000,000 copies/mL) provides a consistent standard across nucleic acid amplification technology (NAT) methods, enabling diagnostic laboratories and *in vitro* Diagnostics (IVD) manufacturers to assess the linearity and accuracy of quantitative NAT systems for the detection of hepatitis B virus (HBV) in plasma samples. This product can be used with amplification methods, including (real time) polymerase chain reaction (PCR) and transcription mediated amplification (TMA) assays. It also can be used as an independent calibration panel for quantification of HBV-DNA concentrations in donor or patient samples. This product is for research use only.

Key to Symbols Used

Manufacturer

Lot number

Catalogue number

Store below -30°C

Research use only

Biological substance category B

Date of manufacturing

Contents

Caution

Read instructions for use

Summary and explanation

In the early 1990s the Eurohep and VQC-Sanquin HBV genotype A standards were the first reference materials used for evaluation of NAT methods[1-4]. Thereafter the Eurohep standard was used for preparation of the 1st and 2nd WHO (97/746 and 97/750) standards[5]. The Eurohep and VQC-Sanquin HBV genotype A standards were independently quantified in equivalent nucleic acid copies. The VQC-Sanquin standard has also been extensively calibrated against the 1st and 2nd lyophilised WHO (97/746 and 97/750) standards and the conversion factors (95% CI) were established at 5.33 (5-11-5.55) and 5.20 (4.61-5.80) copies per IU respectively. The VQC-Sanquin HBV genotype A standard was also calibrated against a chimpanzee plasma of known infectivity[6] and according to this experiment the 50% chimpanzee minimum infectious dose (range) was determined at 4.0 (1.3-12.6) HBV-DNA copies or virions. The S0011 VQC-Sanquin HBV-DNA genotype A standard was used for preparation of a linearity panel composed of 10-fold dilutions ranging from 10,000,000 to 10 copies/mL. The dilutions were made in human citrate plasma to which EDTA was added in order to mimic the matrix of real patient samples. Since this S0011 standard has been extensively calibrated in both copies and IUs[7] it can be used as an independent linearity panel for testing the accuracy and precision of quantitative HBV NAT methods.

Traceability to HBV-DNA copies and International Units

Figure 1 shows the traceability chain between the P0041 HBV-DNA linearity panel, the S0011 VQC-Sanquin HBV-DNA genotype A standard and the 1st and 2nd WHO 97/746 and 96/750 HBV genotype A International Standards.

The viral concentration in the S0011 VQC-Sanquin HBV-DNA genotype A standard was established by laboratories testing dilutions of these standards in the VQC proficiency

program organized between 1996 and 2004. Table 1 compares the geometric mean values in copies/mL as reported by five quantitative NAT methods. It was decided to use the Siemens bDNA 3.0 assay as the reference method for quantification[8] and assign the value of 2.15 x 10^9 copies/mL to the undiluted S0011 VQC-Sanquin standard.

Table 1: Quantification of S0011 VQC-Sanquin HBV-DNA standard in proficiency studies performed between 1996 and 2004. The quantification in the Siemens bDNA 3.0 assay was chosen as the reference method for calibration in copies/mL

Assay	n	copies/mL (95% CI)	Accuracy (95% CI) %
Chiron bDNA 1.0	17	3.22 (3.13-3.32) x 10^9	150 (146-154)%
Siemens bDNA 3.0	**28**	**2.15 (2.11-2.20) x 10^9**	**100 (98-102)%**
Roche Amplicor Monitor	198	2.11 (2.05-2.17) x 10^9	98 (95-101)%
Roche Taqman	8	2.38 (1.01-5.61) x 10^9	111 (47-261)%
Digene HCS	42	1.63 (1.57-1.69) x 10^9	76 (73-79)%

Dr. T. Cuijpers and Dr. M. Koppelman (Sanquin, Amsterdam, the Netherlands) tested dilutions of 1:100, 1:1000, 1:10,000 and 1:30,000 of the S0011 VQC-Sanquin genotype A standard in 4 replicates against dilutions of 1:10 and 1:100 of the WHO 97/746 standard in 6 replicates in the same bDNA 3.0 test run and found a conversion factor (95%CI) of 5.33 (5.11-5.55) copies per IU. Later a 1:66667 dilution of the VQC standard was tested against a 1:543.2 dilution of the WHO 97/750 standard in 6 replicates in the same bDNA 3.0 test run and a conversion factor of 5.20 (4.61-5.80) copies per IU was found. It must be emphasized that the conversion factor from copies to IU values has not yet been established for the 3rd WHO 10/264 replacement standard.

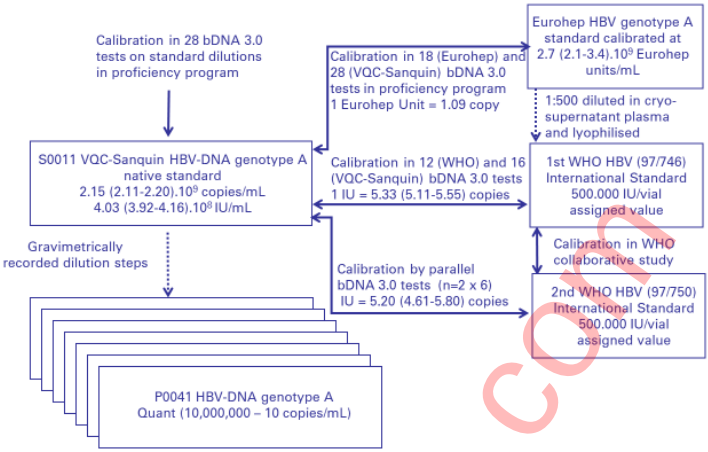
Dilutions of the S0011 VQC-Sanquin HBV genotype A standard were calibrated against those of the Eurohep genotype A standard in the VQC proficiency studies. One copy assigned to the Eurohep standard by Heerman et al[1] was found to be equivalent to 1.09 bDNA copy assigned to the VQC-Sanquin standard.

The S0011 HBV-DNA genotype A standard dilutions in the P0041 linearity panel were made in human plasma using large volume gravimetrically recorded dilutions to guarantee the traceability to copy numbers and IU values.

Calibration of chimpanzee plasmas of known infectivity in copies/mL

An HBV infected chimpanzee plasma taken in the early ramp up phase of viremia was kindly obtained from prof Yoshizawa and Tanaka (Hiroshima University, Japan). According to interpretation of the chimpanzee infectivity data reported by Komiya et al[6] the 50% chimpanzee minimum infectious dose or CID_{50} (and 0% to 100% infectivity range) is 8.2 (2.6-26) copies for the genotype A plasma [C246-P57]. This estimate was based on quantification in the Roche TaqMan assay. Recalibration of a 1:100 dilution of this ramp up phase plasma sample P57 of chimpanzees C-446 in 6 replicate bDNA tests against the VQC-Sanquin genotype A standard showed a conversion factors of 2.06 from bDNA to TaqMan copies for genotype A. As a consequence one CID_{50} (0% to 100% infectivity range) of the HBV genotype A strain contains 4.0 (1.3-12.6) copies when calibrated against the S0011 Sanquin-VQC genotype A standard.

Figure 1. Traceability chain between P0041 HBV-DNA genotype A linearity panel, S0011 VQC-Sanquin standard and WHO International Standards



Stability of HBV standards and reference panels

The long term stability of the liquid frozen HBV standard stored at ≤65°C has been firmly established[9]; hence the stock solutions from which the reference panels are prepared have shown to be stable for more than two decades in the BQC storage facilities. Real time stability experiments using quantitative NAT assays showed no degradation of HBV-DNA reference panels and controls when stored at -30°C[9]. Hence, it can be guaranteed that the reference panels are stable when stored at -30°C.

Materials Provided

Seven (7) panel members; polypropylene tubes (7 mL) with screw caps, each containing 1.2 mL of plasma. The composition is given in table 2

Table 2 Composition of P0041 HBV-DNA genotype A linearity panel. Quantification in copies/mL and calibration in IU/mL against the 1st WHO standard 97/746) was performed on S0011 standard dilutions in multiple replicate bDNA assays (table 1, figure 1).

Panel member	HBV-DNA copies/mL (95 % CI)	HBV-DNA IU/mL (95% CI)	Quantity (mL per vial)
1	$1.00\ (0.98\text{-}1.02).10^{7}$	$1.88\ (1.82\text{-}1.94).10^{6}$	1 x 1.2 mL
2	$1.00\ (0.98\text{-}1.02).10^{6}$	$1.88\ (1.82\text{-}1.94).10^{5}$	1 x 1.2 mL
3	$1.00\ (0.98\text{-}1.02).10^{5}$	$1.88\ (1.82\text{-}1.94.10^{4}$	1 x 1.2 mL
4	$1.00\ (0.98\text{-}1.02).10^{4}$	$1.88\ (1.82\text{-}1.94.10^{3}$	1 x 1.2 mL
5	$1.00\ (0.98\text{-}1.02).10^{3}$	$1.88\ (1.82\text{-}1.94.10^{2}$	1 x 1.2 mL
6	$1.00\ (0.98\text{-}1.02).10^{2}$	$1.88\ (1.82\text{-}1.94.10^{1}$	1 x 1.2 mL
7	$1.00\ (0.98\text{-}1.02).10^{1}$	$1.88\ (1.82\text{-}1.94.10^{0}$	1 x 1.2 mL

Materials not provided

Pipettes or pipetting devices for use in IVD test systems.

Storage instructions

The linearity panel should be stored at or below -30°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

P0041 HBV-DNA Quant (10 to 10,000,000 copies/mL) contains infectious HBV 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[10].

- 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 reference panel is handled.
- Disinfect liquids, materials or spills with a 0.5% sodium hypochlorite solution or equivalent.
- Dispose of all materials and liquids used in the procedure as if they contained pathogenic agents.

Test procedure

- 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 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 a panel member is tested multiple times it should be organized within 8 hours. When not placed in the robot store at 2-8°C.

Interpretation of Results

Precision

Quantitative HBV-NAT methods report Ct values and/or HBV-DNA concentration in either copies/mL or IU/mL. The dilution factor 10 between the subsequent panel members is exact (with 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 other quantitative results one should apply 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 previously used bDNA 3.0 assay as reference method[8]. The IU/mL values are directly traceable to the 1st International Standard (97/746)[5]. The accuracy and precision of copy numbers reported by quantitative HBV-NAT methods used two decades ago is given in the table 1.

Limitations

- P0041 HBV-DNA genotype A Quant (10 to 10,000,000 copies/mL) must not be substituted for the mandatory controls or calibrators provided with quantitative NAT test kits for calculating the lower limit of quantification, the HBV-DNA concentrations and/or criteria for releasing test results.
- The assigned quantification in IU/mL is traceable to the 1st WHO HBV-DNA 97/746 standard and was confirmed in calibration experiment against the 2nd WHO 97/750 standard. The S0011 VQC-Sanquin HBV genotype A standard has not been calibrated against the 3rd WHO 10/264 standard. Therefore theP0041 HBV-DNA genotype A (10 to 10,000,000 copies/mL) linearity panel should not be used for establishing accuracy of quantitative NAT results expressed in IU/mL assigned to the 3rd WHO standard. For this purpose only dilutions of the WHO 10/264 International Standard can be used.
- The panel is for research use only

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