Bio QControl

P0141
HBV 1000 copies/mL genotype reference panel

RUO

REF P0141

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 P0141 Hepatitis B virus (HBV) 1000 copies/mL genotype reference panel enables IVD manufacturers, clinical virology or blood screening laboratories to assess the genotype detection efficiency of nucleic acid amplification test (NAT) methods for the qualitative and quantitative detection of HBV-DNA in plasma samples. This reference panel can be used with amplification methods, including real time PCR and TMA assays and is useful for development and validation of NAT systems. This product is for research use only.

Summary and Explanation

The P0141 HBV 1000 copies/mL genotype reference panel is designed for evaluating the accuracy of quantitative NAT methods (or analytical sensitivity of qualitative NAT methods) in detecting different HBV genotypes. In total 21 different secondary HBV genotype standards that have been extensively characterized in different NAT methods[1,2,3] and 4 negative controls are included in the reference panel (see Table with panel composition below). The range of genotypes covers most of the subgenotypes that are prevalent around the world. The concentration in all panel members is standardized to 1000 copies/mL (approximately 190 IU/mL) as cross calibrated in multiple parallel Siemens Versant bDNA 3.0 assays[4]. This level is far enough above the quantification limit of sensitive viral load assays to obtain consistent quantitative results. The reference panel helps ensure that NAT methods for HBV-DNA detection are properly validated. The HBV standards have been diluted in a pool of plasma units that tested negative for the regular viral markers in individual donation NAT and serology screening assays. The viral concentrations in the plasma pools are ensured by gravimetrically recorded dilutions from calibrated viral stock solutions stored at –30°C.

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 has disappeared. Immediately after thawing, vortex briefly, and give a short spin before releasing screw cap from the vials. The panel members should be handled and tested in a manner identical to that required for clinical specimens run in the test method being evaluated. Follow the manufacturers or laboratory instructions and recommendations for the handling and testing of clinical specimens.

Key to Symbols Used

Date of manufacturing

Manufacturer

Lot number

Catalogue number

Research Use Only

Store at -30°C

Biological substance category B

Expiry date

Contents

Caution

Read instructions for use

Materials Provided

25 x 4.3 mL plasma samples filled off in polypropylene tubes with screw cap divided in 3 parts

Materials not provided

Pipettes or pipetting devices to be used for IVD test systems

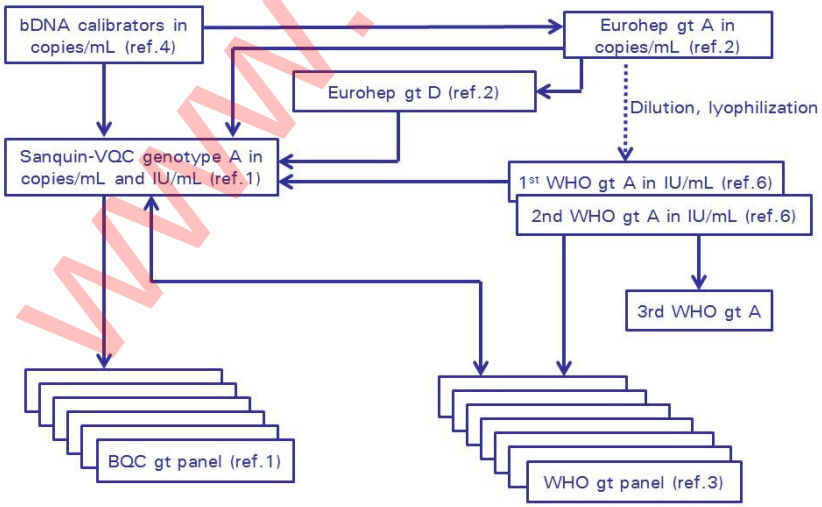
Composition of HBV genotype reference panel

Sample nr.	Secondary Standard[1,2,3]	HBsAg serotype	Origin	copies/mL (95% CI)
1	Sanquin-VQC HBV-DNA genotype A1	adw2	Netherlands	1000 (982-1023)
2	BQC HBV-DNA genotype B1	ayw1	Indonesia	1000 (835-1201)
3	BQC HBV-DNA genotype C1	adr	USA	1000 (846-1181)
4	BQC HBV-DNA genotype D1	ayw2	USA	1000 (852-1173)
5	BQC HBV-DNA genotype E1	ayw3	USA	1000 (904-1108)
6	BQC HBV-DNA genotype F1	adw4	USA	1000 (642-1559)
7	BQC HBV-DNA genotype G1	adw2	USA	1000 (831-1203)
8	Eurohep HBV-DNA genotype A21,2	adw2	Germany	1000 (599-1667)
9	Eurohep HBV-DNA genotype D1,2	ayw2/3	Germany	1000 (803-1253)
10	WHO HBV-DNA genotype A13	adw2	South Africa	1000 (632-1583)
11	WHO HBV-DNA genotype A13	adw2	Brasilia	1000 (531-1885)
12	WHO HBV-DNA genotype A23	adw2	Germany	1000 (833-1200)
13	WHO HBV-DNA genotype B13	adw2	Japan	1000 (642-1556)
14	WHO HBV-DNA genotype B23	adw2	Japan	1000 (673-1485)
15	WHO HBV-DNA genotype C23	adr	Japan	1000 (678-1453)
16	WHO HBV-DNA genotype C23	adr	Japan	1000 (728-1373)
17	WHO HBV-DNA genotype C23	adr	Russia	1000 (734-1362)
18	WHO HBV-DNA genotype D13	ayw2	Germany	1000 (634-1573)
19	WHO HBV-DNA genotype D33	ayw2	South Africa	1000 (802-1245)
20	WHO HBV-DNA genotype D13	ayw3	Iran	1000 (657-1524)
21	WHO HBV-DNA genotype E3	ayw4	West Africa	1000 (794-1258)
22	Negative human plasma			
23	Negative human plasma			
24	Negative human plasma			
25	Negative human plasma			

Calibration of secondary HBV standards and traceability to WHO International Standard

The Figure summarizes calibration studies of different secondary HBV standards. Grabarczyk et al[1] describe (in Supplemental materials available at the Transfusion website) that the Sanquin-VQC HBV-DNA genotype A standard has been quantified in 28 bDNA assays at 2.15 (2.11-2.22) x 10^9 copies/mL and in similar concentrations by other quantitative HBV-DNA assays. The Sanquin-VQC genotype A standard has been calibrated against the WHO 97/746 standard using multiple bDNA3.0 assays and two independent experiments showed conversion factors (95% CI) of 5.33 (5.11-5.55) and 5.20 (4.61-5.80) copies/IU respectively[1], whereas a conversion factor of 4.12 copies/IU (with wide confidence limits) can be calculated from the WHO collaborative study[6] in which the Sanquin-VQC standard was represented as candidate ZZ. All BQC genotype B-G standards as well as the Eurohep genotype A and D standards were calibrated against the Sanquin-VQC standard in 6 to 12 parallel bDNA 3.0 assays and these experiments showed maximum confidence intervals of 60% to 170% of the established concentration in copies/mL[1]. These calibration experiments confirmed the concentrations established by Heerman et al[2] with conversion factors of 1.10 and 1.00 bDNA copy/Eurohep copy for the Eurohep genotype A and D standards respectively[1]. More recently Chudy et al[3] have established the WHO HBV genotype reference panel and lyophilized samples of the highest concentrations were included in the BQC HBV 1000 cps/mL genotype reference panel (see Table below). Dilutions of the WHO HBV genotype reference samples and the Sanquin-VQC standard have been tested in 6 parallel bDNA 3.0 assays, which confirmed the bDNA assay results reported in the WHO collaborative study. Chudy et al[3] found some variation in quantitative results on genotypes in different NAT methods when compared to the WHO 97/746 standard and therefore did not assign IU/mL values to the members of the WHO reference panel. The same stock solutions of the Sanquin, BQC, Eurohep and WHO HBV genotype standards that were used for the bDNA calibration experiments were also used for preparation of the 1000 cps/mL dilutions which were recorded gravimetrically. The 95% confidence intervals found in the bDNA 3.0 assay calibration experiments around the targeted 1000 copies/mL levels in the HBV genotype reference panel are shown in the Table above.

Figure. Traceability of secondary HBV standards



Statistical evaluation of quantitative results

The samples in the P0141 HBV 1000 cps/mL genotype reference panel have been carefully formulated to mimic human plasma specimens containing 1000 copies/ml of HBV-DNA according to the Siemens Versant bDNA 3.0 assay when calibrated against the Sanquin-VQC genotype A standard (sample 1 in the reference panel). For statistical comparison of quantitative results in viral load assays it is recommended to test the samples in parallel in the same test runs until the required number of replicates per sample is available. In most cases a transformation of quantitative values is required to obtain a normal distributed data-set. After transformation one can use a paired t-test to compare mean values and to identify possible significant differences in genotype detection efficiency. For example, when a real time PCR assay is applied the Cycle to threshold (Ct) values can be used to calculate mean and standard deviation. Ideally the paired t-test should not show a significant difference. If there is a significant difference in quantitative values on the genotype standards this may be indicative for differences in genotype detection efficiency of the assay being evaluated and the bDNA 3.0 assay used to establish the 1000 cps/mL HBV reference panel.

Precautions

Warning: The HBV-DNA genotype panel members contain infectious HBV particles and are bio-hazardous. Observe the universal precautions for prevention of transmission of infectious agents when handling these materials[7,8,9]. Although the normal human plasma used in the production of this panel was negative for infectious disease markers the reference 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 specimens are 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.

Storage Instructions

It is recommended that the panel is stored at –30°C or lower to ensure highest quality. Discard any unused material after the first use. Any panel members that appear cloudy or contain visible precipitates after thawing should be discarded.

Limitations

The P0141 1000 cps/mL HBV genotype reference panel is not intended to replace the internal calibrators integral to in vitro diagnostic (IVD) test kits, but may be used as external, independent secondary or tertiary standards for the assessment of the performance of qualitative or quantitative NAT assays. A significant difference between the quantitative values assigned to the one or more members of this panel and those found by the NAT method evaluated could origin from an underestimation or overestimation of the viral load for certain genotypes by the assay under investigation. However BioQControl makes no warranty of any kind as to the suitability of this panel for the proper assessment of genotype detection efficiency of NAT systems.

References

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