Bio QControl

P0153
HAV 100 IU/mL
genotype reference panel

RUO

REF P0153

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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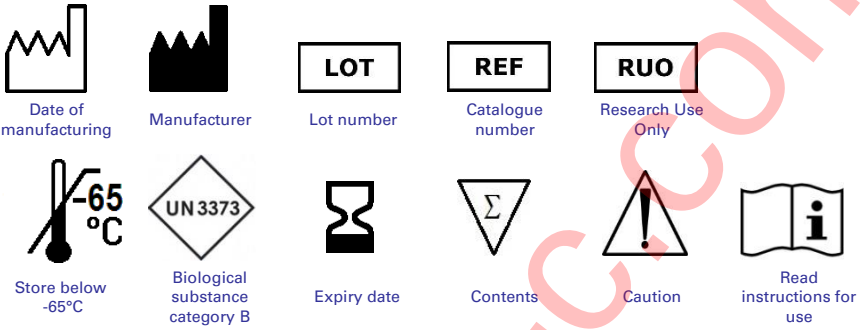
Intended Use

The P0153 Hepatitis A virus 100 IU/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 HAV-RNA 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.

Key to Symbols Used



Summary and Explanation

The P0153 HAV 100 IU/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 5 different AV genotype standards are included in the reference panel (see Table 1 with panel composition below). The available range of genotypes covers most of the sub-genotypes that are prevalent around the world. The concentration in all panel members is standardized to 100 IU/mL. 2 panel members are dilutions of the second and third WHO International standard, while the other three standards were (in)direct calibrated on the first WHO International standard[1,2,3] using real time PCR. The concentration of 100 IU/ml is well above the quantification limit of sensitive viral load assays to obtain consistent quantitative results. The reference panel helps ensure that NAT methods for HAV-RNA detection are properly validated. The HAV standards have been diluted in a pool of plasma units that tested negative for the regular viral markers in individual donation NAT screening assays. The viral concentrations in the plasma pools are ensured by gravimetrically recorded dilutions from calibrated viral stock solutions stored below –65°C.

Kit contents (materials provided)

The run control contains human plasma without preservatives and is provided as detailed in Table 1.

Table 1. Description of kit formats and contents

Cat. Code	Description of contents	Primary packing	Secondary packing
P0153	5 x 4.0 mL panel member	10 mL vial	Plastic zip bag

Materials required but not supplied

The test kits and liquid handling devices provided by the NAT manufacturer

Composition of HAV genotype reference panel

Table 1 panel composition

Sample nr.	Secondary Standard	Origin	IU/mL
1	Sanquin-VQC HAV-RNA genotype 1a	The Netherlands	100
2	2nd WHO standard genotype 1a[1,2]	United Kingdom	100
3	3rd WHO standard genotype 1b[3]	United Kingdom	100
4	BQC HAV-RNA genotype 2a	France	100
5	BQC HAV-RNA genotype 3a	France	100

The quantification of sample 2 and 3 is exact; calculated from the respective WHO standards and sample 1, 4 and 5 are calibrated on the 1st WHO standard[1].

Calibration of secondary HAV standards and traceability to WHO International Standard

Figure 1 summarizes the calibration and manufacturing relations between the standards used in this panel. The preparation and calibration trail of different primary and secondary HAV standards starts with the study for establishing the first WHO International standard. In this study the 1st, 2nd WHO International standard and the BioQControl primary standards were calibrated on each other. All WHO standards are prepared from a diluted plasma unit while the BioQControl standards are tissue cultures diluted in human plasma.

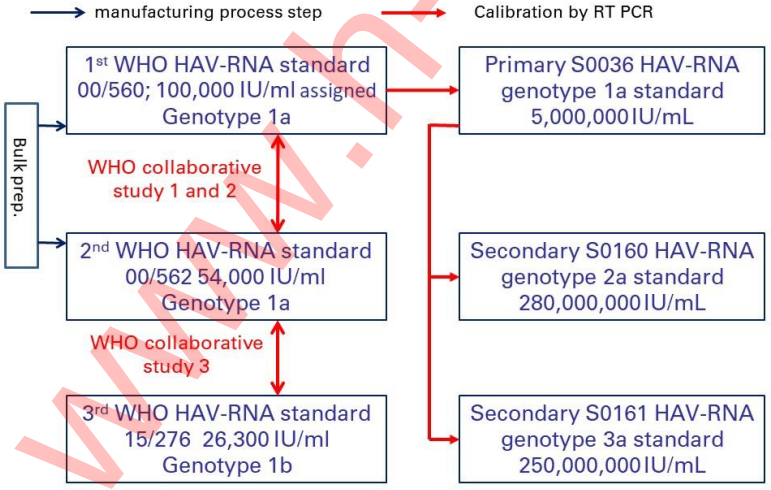


Figure 1 relationship HAV-RNA standards used in P0153

Following the assigned value for the 1st WHO standard we established S0036 at 5.000.000 IU/ml. In the second[2] and third WHO study[3] the respective values of the second and third WHO International standards were confirmed, assigned. The values for S0160 and S0161 were found by multiple testing dilution series against S0036 in a real time in house PCR[4].

Relationship IU and copy

Both the 1st WHO HAV-RNA International standards and BioQControl S0036 primary standard were used to verify the analytical sensitivity of blood screening tests. The results of these studies are used to relate the International Unit to NAT detectable units/ml. When we assume the tests are ideal this is also equivalent to copies/ml. Testing in the Grifols test resulted in the highest conversion factors, while we found lower results for Roche DPX[5]. In total 5 Grifols studies confirmed each other conversion factor. The results are summarized in table 2.

Table 2 overview results limiting dilution series

Source	63 % hit rate	IU to copy	standard
URBM EFS	0.24 (0.18-0.33)	8.2 (6.2-11.2)	00/560
Poland PI	0.25 (0.22-0.29)	8.0 (6.8-9.2)	00/560
Dev. Lot data Grifols	0.23 (0.16-0.36)	8.8 (5.2-13.0)	00/560
Finnish red cross#	0.19 (0.05-0.36)	10.4 (2.8-22)	00/560
Dev. Lot data Grifols	0.28 (0.06-1.17)	7.0 (1.7-33)	S0036

series were not complete.

We conclude a conversion factor of 8.4 is the best estimate available. Please consider this informative as quantitative tests including SI traceable calibrators are lacking.

Statistical evaluation of quantitative results

The samples in the P0153 HAV 100 IU/mL genotype reference panel have been carefully formulated to mimic human plasma specimens containing 100 IU/ml of HAV-RNA. For statistical comparison of quantitative results in viral load assays it is recommended to test the samples in parallel in the same test run until the required number of replicates per sample is available. In most cases a log transformation of quantitative values or Ct value is required to obtain a normal distributed dataset. 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.

Warning and precautions

The P0153 HAV 100 IU/ml genotype reference panel members contain infectious HAV virions and are bio hazardous. Observe the universal precautions for prevention of transmission of infectious agents when handling these materials[6,7]. 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 subtype 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.

- 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 P0153 HAV 100 IU/ml genotype reference panel and 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.

Reagent preparation

- 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 procedure being evaluated.
- Follow the manufacturers or testing laboratory instructions and recommendations for the handling and testing of clinical specimens.

Storage Instructions

It is recommended that the panel is stored at –65°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 P0153 100 IU/mL HAV 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 first, secondary or tertiary standard panel 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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2. Fryer JF, Heath AB, Morris CL and the collaborative study group. Collaborative study to evaluate the proposed 2nd WHO International Standard for hepatitis A virus (HAV) for nucleic acid amplification technology (NAT)-based assays. WHO ECBS Report 2013; WHO/BS/2013.2225.

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