Bio QControl

HAV-RNA reference panels

RUO

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 HAV-RNA panels for sensitivity analysis

This insert describes the panels that can be used to establish the analytical sensitivity of HAV-RNA assays. Table 1 presents an overview of the available panels of different genotypes.

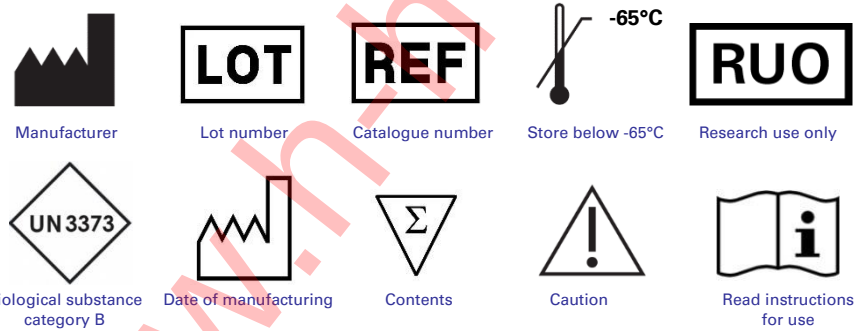
Table 1. Product overview: HAV-RNA reference panels

Catalogue nr.	Product name	number samples
P0351	P0351 HAV-RNA genotype 1a strain HM175/18F	8
P0208	P0208 HAV-RNA genotype 2a	8
P0209	P0209 HAV-RNA genotype 3a	8

Intended Use

The HAV-RNA reference panels are intended for determining the analytical sensitivity of molecular test procedures for the qualitative and quantitative detection of Hepatitis A virus (HAV) RNA in plasma samples. The panels can be used with nucleic acid amplification technology (NAT) methods, including TMA and real-time PCR assays. The HAV-RNA reference panels are useful for establishing the 95% and 50% limit of detection (LOD) and limit of quantification (LOQ). They could also be used for batch acceptance, NAT system validation and training of technicians. The products are for research use only and not for diagnostic use.

Key to Symbols Used



Summary and explanation

The HAV-RNA reference panels are designed for testing the analytical sensitivity or quantification limits of NAT methods. The reference panel helps to ensure that NAT procedures for HAV-RNA are properly validated, and that test results are consistent across manufacturers, testing laboratories, operators, platforms and assay formats. All HAV-RNA standards are tissue culture supernatants. The quantification of panel members is expressed in IU/mL and copies/mL. The quantification of the HAV-RNA genotype 1a standard in IU/mL is based on calibration against the first WHO International Standard (NIBSC code 00/560) using real time PCR[1,2]. The quantification in copies/mL originates from limiting dilution analysis using the Grifols Procleix HAV/Parvo B19V assay. From these data 1 IU is equivalent to approximately 10 HAV-RNA copies (with a considerable uncertainty range). The calibration of HAV-RNA genotype 2a and 3a standards is performed by real time PCR[3] testing against the HAV-RNA genotype 1a standard.

For preparation of the reference panels the HAV-RNA standards are diluted in a pool of plasma units that tested negative for viral markers by NAT and serologic testing. Viral stock solutions and reference panels are snap frozen in liquid nitrogen prior to storage at ≤ -65°C. Lot-to-lot consistency of the viral concentrations in the reference panels is ensured by gravimetrically recorded dilutions from calibrated viral stock solutions, stored at ≤ -65°C and batch release testing using real time PCR.

Materials Provided

Eight (8) polypropylene tubes (10 mL) with screw caps (8 members), containing 4.0 mL. Table 2 presents the quantification of the panel members, listed in table 1.

Table 2. Quantification of te panel members.

Catalogue. Nr.	Member-id	IU/mL	~ copies/mL
P0351	B4300-xxx-01	30	300
P0351	B4300-xxx-02	10	100
P0351	B4300-xxx-03	3	30
P0351	B4300-xxx-04	1	10
P0351	B4300-xxx-05	0.3	3
P0351	B4300-xxx-06	0.1	1
P0351	B4300-xxx-07	0.03	0.3
P0351	B4300-xxx-08	0.01	0.1
P0208	B4208-xxx-01	300	3000
P0208	B4208-xxx-02	100	1000
P0208	B4208-xxx-03	30	300
P0208	B4208-xxx-04	10	100
P0208	B4208-xxx-05	3	30
P0208	B4208-xxx-06	1	10
P0208	B4208-xxx-07	0.3	3
P0208	B4208-xxx-08	0.1	1
P0209	B4209-xxx-01	300	3000
P0209	B4209-xxx-02	100	1000
P0209	B4209-xxx-03	30	300
P0209	B4209-xxx-04	10	100
P0209	B4209-xxx-05	3	30
P0209	B4209-xxx-06	1	10
P0209	B4209-xxx-07	0.3	3
P0209	B4209-xxx-08	0.1	1

The tube identification is Byyyy-xxx-number, where yyyy is product specific and xxx the sequential batch number. The identification is present on the bar-code and further explained on the tube label.

Materials not provided

Pipettes or pipetting devices for use in IVD test systems.

Storage Instructions

It is recommended that the panel is stored at ≤ –65°C 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 HAV-RNA reference panel members contain infectious HAV and are potentially bio-hazardous. Apply the universal precautions for prevention of transmission of infectious agents when handling these materials[4]. Although the normal human plasma used in the production of this panel was negative for blood borne viruses 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 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.

Interpretation of Results

Limit of detection (LOD)

For establishing the NAT detection limit the panel should be tested in multiple replicates. It is recommended to test panel members at least 12 times. The proportion of reactive results can then be interpreted using probit analysis[5]. For estimating he 95% and 50% LOD reliably standard dilutions with 100% reactivity down to below 50% reactivity should be included in the analysis. Apply the log transformation on the concentration in IU/mL before using the probit analysis.

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 suitable precision and accuracy.

Limitations

The concentrations in copies/mL should not be used for accuracy analysis in quantitative assays. The concentrations in IU/mL are traceable to the 1st WHO 00/560 standard and not to later WHO HAV-RNA replacement standards.

References

1. Saldanha J, Heath A, Lelie N, Pisani G, Yu MY; Collaborative Study Group. A World Health Organization International Standard for hepatitis A virus RNA nucleic acid amplification technology assays. Vox Sang. 2005;89:52-8.
2. WHO/BS/2013.2225 Collaborative Study to Evaluate the Proposed 2nd WHO International Standard for Hepatitis A Virus (HAV) for Nucleic Acid Amplification Technology (NAT)-Based Assays Jacqueline F. Fryer, Alan B. Heath, Clare L Morris and the Collaborative Study Group
3. Koppelman MH, Cuijpers HT, Wessberg S, Valkeajärvi A, Pichl L, Schottstedt V, Saldanha Multicenter evaluation of a commercial multiplex polymerase chain reaction test for screening plasma donations for parvovirus B19 DNA and hepatitis A virus RNA. Transfusion. 2012;52:1498-508.
4. Centers for Disease Control (CDC). Guidelines for prevention of transmission of human immunodeficiency virus and hepatitis B virus to health-care and public-safety workers. MMWR 1989; 38(S-6): 1-36.
5. Probit Analysis. [2nd ed.] by D. J. Finney

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