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

Table of contents

Overview HAV-RNA panels for sensitivity analysis

This insert describes the following panels which can be used to establish sensitivity in screening assays and determination of accuracy, precision and lower limit of quantification, detection for quantitative HAV-RNA assays. Table 1 present an overview of available panels. All product names provide origin to standard and genotype.

Table 1 product overview

Catalogue nr.	Product name	number samples
P0136	P0136 HAV-RNA genotype 1a strain HM175/18F	10
P0208	P0208 HAV-RNA genotype 2a	8
P0209	P0209 HAV-RNA genotype 3a	8

Intended Use

The HAV-RNA reference panels provide a consistent standard across NAT methods, enabling blood screening laboratories and diagnostic manufacturers to assess the analytical sensitivity and quantification limits of molecular diagnostic test procedures for the qualitative and quantitative detection of Hepatitis A virus (HAV) in blood samples. This product can be used with amplification methods, including TMA and real-time PCR assays and is useful for testing the analytical sensitivity, LOQ, LOD and qualification of new diagnostic kit lots or NAT system validation and training. It also can be used as a calibration panel in quantification of low HAV-RNA concentrations in the window phase. The products are research use only.

Key to Symbols Used


Manufacturer

Lot number

Catalogue number

Store below -65°C

Research use onl

Biological substance category B


Date of manufacturing

Contents

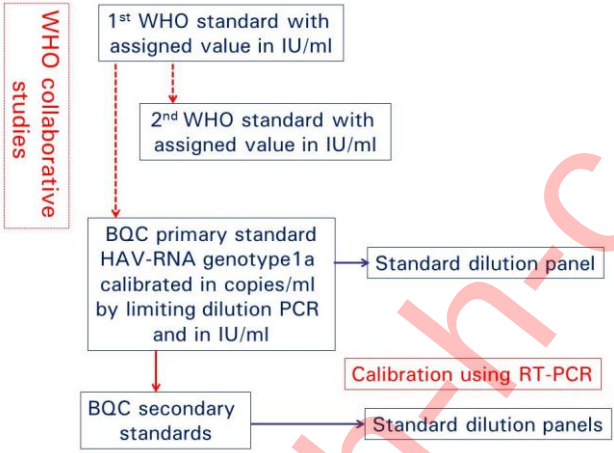

Caution


Read instructions f use

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 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. Figure 1 present the relationship between the different standards[1,2,3] which were used for calibration and manufacturing of the panels. The quantification is expressed in IU/ml and copies/ml. The quantification in IU/ml is found by calibration on the first WHO International Standard using Real Time PCR while the quantification in copies/ml originates from limiting dilution analysis using the Grifols HAV/Parvo Duplex assay on HAV-RNA genotype 1a dilution series. The calibration of secondary HAV-RNA standards is done by RT-PCR[4].

Figure 1; calibration relation between different HAV-RNA reference panels.



The HAV-RNA reference panels are designed for testing the analytical sensitivity or quantification limits of NAT methods. The reference panels help ensure NAT procedures for HAV-RNA are properly validated, and test results are consistent across manufacturers, testing laboratories, operators, platforms and assay formats. The HAV-RNA reference panels were prepared from characterised HAV-RNA tissue culture plasma standards.

Principles of the Evaluation Procedure

HAV-RNA reference panel members have been carefully formulated to mimic human plasma specimens containing low concentrations of HAV-RNA. The HAV-RNA reference panels are suitable for replicate testing and determination of the 95% and 50% detection limits of the NAT blood screening systems by probit analysis[5]. The panels are also suitable for testing the lineairity and, or lower quantification limit of viral load assays, such as real time PCR.

Materials Provided

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

Cat. Nr.	member-id	IU/ml	copies/ml (95 % C.I.)
P0136	B4136-xxx-01	1420	14200
P0136	B4136-xxx-02	710	7100
P0136	B4136-xxx-03	142	1420
P0136	B4136-xxx-04	71	710
P0136	B4136-xxx-05	14.2	142
P0136	B4136-xxx-06	7.1	71.0
P0136	B4136-xxx-07	1.42	14.2
P0136	B4136-xxx-08	0.71	7.1
P0136	B4136-xxx-09	0.142	1.42
P0136	B4136-xxx-10	0.071	0.71
P0208	B4208-xxx-01	213	2130
P0208	B4208-xxx-02	71.0	710
P0208	B4208-xxx-03	21.3	213
P0208	B4208-xxx-04	7.10	71.0
P0208	B4208-xxx-05	2.13	21.3
P0208	B4208-xxx-06	0.71	7.1
P0208	B4208-xxx-07	0.23	2.3
P0208	B4208-xxx-08	0.071	0.71
P0209	B4209-xxx-01	213	2130
P0209	B4209-xxx-02	71.0	710
P0209	B4209-xxx-03	21.3	213
P0209	B4209-xxx-04	7.10	71.0
P0209	B4209-xxx-05	2.13	21.3
P0209	B4209-xxx-06	0.71	7.10
P0209	B4209-xxx-07	0.23	2.30
P0209	B4209-xxx-08	0.071	0.71

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 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 HAV-RNA reference panel members contain infectious HAV and are potentially bio-hazardous. Observe the universal precautions for prevention of transmission of infectious agents when handling these materials[6]. 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 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 refreeze panel members after thawing. In that case we cannot guarantee the claims given.

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.
- Vortex the run control.
- Give a short spin in a centrifuge before releasing screw cap from vial.
- Minimise the time period from thawing until usage of the members, you can store up to 8 hours at 2-8°C[5].
- The panel member should be handled and tested in a manner identical to that of clinical specimens in the test procedure being evaluated.

Expected assay response values

The expected quantitative results are given in table 2. The lowest concentrations are beyond the detection limit, or lower limit of quantification. These concentration will not react in all cases positive, or yield a quantitative result.

Interpretation of Results

Limit of detection

Establishing the detection limit for screening assays is done by testing the whole panel multiple times, we recommend at least 12 times all concentrations below 100 % reactivity reactivity. The proportion positive or negative results are intepreted using probit analysis[5]. For an correct outcome both results above and below 50 % positive, and at least two concentrations with intermediate reactivity should be available. Apply the log transformation on the concentration before using the probit analysis. You should report both the 50 and 95 % hit rate for intepretation by third parties. The limit of detection is often defined as the 95 % hit rate.

Limit of quantification; precision and accuracy

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

- **Checking amplification efficiency**.
  For nucleic acid test a relation between log(concentration) and log(quantitative results) 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 on the slope overlaps -1.00.
- **Calculation of precision**.
  The accuracy around the LOQ becomes less. One should calculate for each measurement the - ^{2}log(concentration) + ^{2}log(result), ^{2}log(result) can be replaced by –Ct value. For each concentration determine the average and standard deviation of the sum. The cumulative Chi-square distribution is used to compare the probability the SD of one concentration (s) is significantly different from the SD of all concentrations included:
  n is number of measurements
  Calculate SD on the log (concentration) or Ct value within one concentration evaluated (n>10): $s^{2`}$
  Calculate SD on the log (concentration) or Ct value of the reference period: σ^2
  Calculate $X^2=(n-1)\ s^2/\sigma^2$

Table 4. Chi-square (X^2) values for p=0.05

n-1(df)	X^2	n-1 (df)	X^2	n-1 (df)	X^2
11	19.69	21	32.67	40	55.76
12	21.01	22	33.92	50	67.51
13	22.36	23	35.17	60	79.08
14	23.69	24	36.42	70	90.53
15	25.00	25	37.65	80	101.88
16	26.30	26	38.89	90	113.15
17	27.59	27	40.11	100	124.34
18	28.87	28	41.34		
19	30.14	29	42.56		
20	31.41	30	43.77		

Interpretation:
Chi-square: X^2(Calculated) <X^2(P=0.05) : SD is not significantly changed.
Chi-square: X^2 (Calculated) ≥X^2 (P=0.05) : SD has changed significantly.

For concentrations with SD's not significantly differing, the average SD on the sum is calculated. When not use the SD per concentration

Table 4. Relation of Student t value and numbers of measurements (n) to calculate CI's.

Run (n)	t-value at 95% C.I.	t-value at 99% C.I.
10	2.306	3.355
20	2.101	2.878
30	2.048	2.763
infinite	1.960	2.576

The lower limit (%) =$10^{(-t\text{-value x SD})}$ and higher limit (%)=$10^{(t\text{-value x SD})}$

Calculation of accuracy
Use all concentrations with an equal SD. Calculate delta = Log(concentration assigned) log(concentration measured) for each measurement. The accuracy = $10^{-\text{average delta}}$

On our website www.biogcontrol.com excell spreadsheets for performing the calculations are made available.

Limitations

The concentrations in IU/ml and copies/ml should not be used for accuracy analysis on quantitative assays.

References

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