Parvo B19-DNA 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 Parvo B19-DNA 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 Parvo B19-DNA assays. Table 1 present an overview of all available panels. All product names provide origin to standard and genotype.

Table 1 product overview

Catalogue nr.	Product name	number samples
P0143	P0143 Parvo B19-DNA genotype 1	10
P0144	P0144 Parvo B19-DNA genotype 2	9

Intended Use

The Parvo B19-DNA reference panels provide a consistent standard across Nucleid acid testing (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 Parvo B19 virus genotype 1 in blood samples. This product can be used with amplification methods, including TMA and real-time PCR assays and is useful for evaluating the analytical sensitivity, qualification of new diagnostic kit lots or NAT system validation and training. The product is for research use only, not for diagnostic use.

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 Parvo B19-DNA reference panels are designed for testing the accuracy of quantification of NAT methods. The reference panel helps ensure that NAT procedures for Parvo B19-DNA 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[1,2,3,4,5] which were used for calibration. The quantification is expressed in IU/ml. The quantification in IU/ml is found by calibration on the first WHO International Standard using Real Time PCR while the calibration of secondary Parvo B19-DNA standards is done on the genotype 1 standard[7,8]. Parvo B19-DNA testing should be able to detect 10.000 IU/ml in plasma(pools) for fractionation according the Pharmacopeia. Results below this level are considered to be safe fro manufacturing plasma products[8].

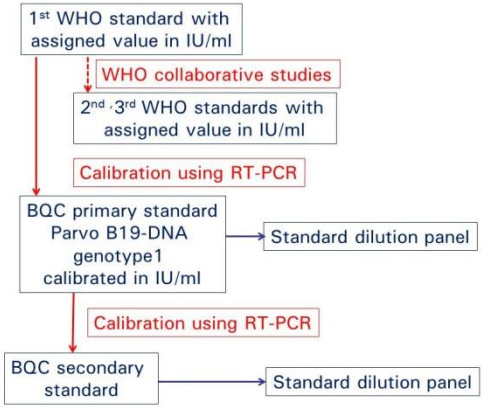

Figure 1; calibration relation between different Parvo B19-DNA reference panels.

The Parvo B19-DNA reference panels are designed for testing the analytical sensitivity or quantification limits of NAT methods. The reference panels help ensure NAT procedures for Parvo B19-DNA are properly validated, and test results are consistent across manufacturers, testing laboratories, operators, platforms and assay formats. The Parvo B19-DNA reference panels were prepared from well characterised Parvo B19-DNA plasma standards[1].

Principles of the Evaluation Procedure

Parvo B19-DNA reference panel members have been carefully formulated to mimic human plasma specimens containing low concentrations of Parvo B19-DNA. The Parvo B19-DNA 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. The panels are also suitable for testing the 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 (9) polypropylene tubes (10 mL) with screw caps (8 or 10 members), containing 4.0 mL.

Cat. Nr.	member-id	IU/ml
P0143	B4143-xxx-01	1.000.000
	B4143-xxx-02	300.000
	B4143-xxx-03	100.000
	B4143-xxx-04	30.000
	B4143-xxx-05	10.000
	B4143-xxx-06	3.000
	B4143-xxx-07	1.000
	B4143-xxx-08	300
	B4143-xxx-09	100
	B4143-xxx-10	30

Cat. Nr.	member-id	IU/ml
P0144	B4144-xxx-01	100.000
	B4144-xxx-02	30.000
	B4144-xxx-03	10.000
	B4144-xxx-04	3.000
	B4144-xxx-05	1.000
	B4144-xxx-06	300
	B4144-xxx-07	100
	B4144-xxx-08	30

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 Parvo B19-DNA reference panel members contain infectious Parvo B19 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 the concentrations with intermediate reactivity. The positive or negative results are intepreted using probit analysis[7]. 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) $\geq X^2$ (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.

References

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