# 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 presents an overview of available panels. All product names provide origin to standard and genotype.

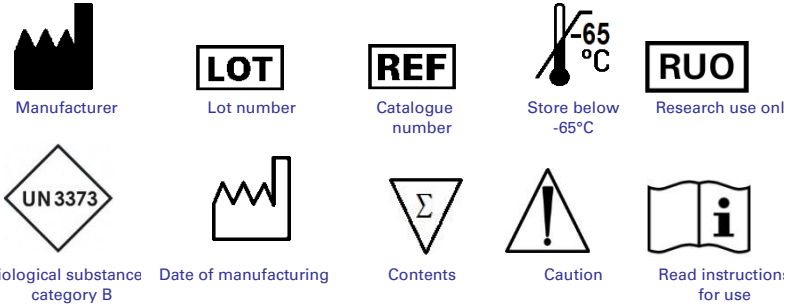
**Table 1** product overview: Parvo B19-DNA reference panels

| Catalogue nr. | Product name | number samples |
|---|---|---|
| P0143 | P0143 Parvo B19-DNA genotype 1 | 10 |
| P0144 | P0144 Parvo B19-DNA genotype 2 | 8 |

## Intended Use

The parvo B19-DNA reference panels provide a consistent standard across NAT methods, enabling blood screening laboratories and diagnostic manufacturers to assess the quantification limits of molecular diagnostic test procedures for the quantitative detection of parvo B19 virus (parvo B19) in blood samples. This product can be used with amplification methods, including TMA and real-time PCR assays. The parvo B19-DNA reference panels are useful for establishing the limit of quantification (LOQ), batch acceptance, NAT system validation and training. The product are research use only and 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 instruction for use

## Summary and explanation

The parvo B19-DNA reference panel helps ensure 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] 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 for Parvo B19-DNA (99/800) using an in house Real Time PCR (Sanquin Blood Supply, department Viral Diagnostic Services) while the calibration of secondary Parvo B19-DNA genotype 2 standard is done on the genotype 1 standard[5,6,7]. 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].

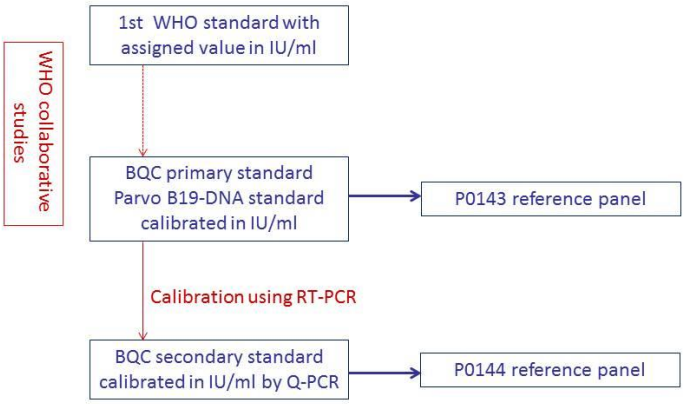



**Figure 1**: calibration relation between different Parvo B19-DNA reference panels.

The Parvo B19-DNA reference panels are designed for testing the quantification limits, precision 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].

## 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. The quantification in IU/ml is obtained using the conversion factor. A confidence interval cannot be given, the quantification originate from the WHO collaborative study. No details on confidence intervals are published.

| Catalogue 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 |

| Catalogue nr. | member-id | IU/ml |
|---|---|---|
| P0144 | B4144-xxx-01 | 330.000 |
| | B4144-xxx-02 | 110.000 |
| | B4144-xxx-03 | 33.000 |
| | B4144-xxx-04 | 11.000 |
| | B4144-xxx-05 | 3.300 |
| | B4144-xxx-06 | 1.100 |
| | B4144-xxx-07 | 330 |
| | B4144-xxx-08 | 110 |

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[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 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. When you intend to test a member multiple times you should organize this within 8 hours duration. When not placed in the robot store at 2-8°C.**

## Interpretation of Results

**Limit of quantification; precision and accuracy**

**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.

- **Checking amplification efficiency**.
  For nucleic acid test the relation between $^2$log(concentration) and $^2$log(quantitative results) or Ct value 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): $s^2$, SD on the log (concentration) or Ct value of the reference members: $\sigma^2$

Calculate $X^2=(n-1)\ s^2/\sigma^2$

**Table 3**. 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.

We advice to report the LOQ as the lowest level having a consistent outcome; SD is not significantly increased.

**Comparison to given concentrations: accuracy.**

For concentrations with SD's not significantly different, the average SD on the sum is calculated. When not use the SD for each concentration and report accordingly.

**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 (%) $=10^{(-t\text{-value} \times SD)}$ and higher limit (%)$=10^{(t\text{-value} \times SD)}$

**Calculation of accuracy**

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

Excel spreadsheets for performing the calculations are made available upon request.

## References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