P0266
ViraQ Parvo B19/HAV Check

CE
0483

IVD

REF P0266

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

P0266 ViraQ Parvo B19/HAV Check is intended to be used as external run control for detection of parvovirus B19 DNA and hepatitis A virus (HAV)-RNA by the assays on the platforms defined in Table 1. P0266 ViraQ Parvo B19/HAV Check is a dual marker mixture of parvovirus B19 DNA and HAV-RNA standards in plasma at a concentration of 10,000 International Units (IU)/mL and 10 IU/mL, respectively, giving a quantitative result on parvovirus B19 DNA and a low reactive result on HAV-RNA in the cobas and Procleix assays. The run control is intended for repeated testing in consecutive test runs of the assays over time by trained laboratory workers. The run control helps laboratories to ensure sufficient analytical sensitivity of HAV-RNA detection and consistent quantification of parvovirus B19 DNA by these nucleic acid amplification technology (NAT) assays used for plasma (mini)pool testing. P0266 ViraQ Parvo B19/HAV Check should not be used to replace the internal controls or calibrators in the test kits. The test result on the run control should not be used to reject the run or delay the release of test results on donor samples.

Table 1. Assays and platforms covered by P0266 ViraQ Parvo B19/HAV Check control.

Assay (manufacturer)	Platform	Test environment
cobas® DPX assay (Roche)	cobas® 5800/6800/8800	Blood screening
Procleix® Parvo/HAV assay (Grifols)	Procleix® Panther®	

Key to Symbols Used

Manufacturer

Lot number

Catalogue number

In vitro diagnostic medical device

Country of origin

Expiry date

Number of controls

Caution

CE mark with Notified Body number

Unique device identification

Biological substance Category B

Store below –30 °C

Positive control

Contains human blood derivatives

Read instructions for use

Principle of method

P0266 ViraQ Parvo B19/HAV Check control has been formulated to mimic plasma pool specimens with 10,000 IU/mL of parvovirus B19 DNA and 10 IU/mL of HAV-RNA. The run control can be used for external quality control of plasma (mini)pool testing runs. After thawing the run control tubes are ready for use and can be placed at random positions in sample racks on the NAT platforms. The run control has been designed to ensure sufficient analytical sensitivity of HAV-RNA detection and consistent quantification of parvovirus B19 DNA by real time polymerase chain reaction (PCR) and transcription

mediated amplification (TMA) tests in blood screening laboratories. The parvovirus B19 DNA concentration in the run control has been set at 10,000 IU/mL because the European Pharmacopoeia (group 6B) and the Food and Drug Administration (FDA) in the US have determined (or recommended) this to be the acceptance limit for the viral load in the manufacturing plasma pools for preparation of certain plasma products[1,2]. To achieve this goal, plasma minipool testing is performed to avoid contamination of the manufacturing pools by HAV or parvovirus B19 concentrations affecting the safety of the plasma products[3,4,5]. The HAV-RNA concentration in P0266 ViraQ Parvo B19/HAV Check control has been set at 7-11 times the 95% lower limit of detection (LOD) of the Roche cobas DPX and Grifols Procleix Parvo/HAV assays (Table 2). The positioning of P0266 ViraQ Parvo B19/HAV Check control for HAV-RNA ensures reactivity rates above 99.9% in the NAT systems listed in Table 1. The run control enables laboratories to be alerted in case of a significant reduction in the analytical sensitivity of HAV-RNA detection or a major change in the quantification of parvovirus B19 DNA by the NAT systems. The run control is a dilution of the native BioQControl S0037 parvovirus B19 DNA genotype 1 and S0036 HAV-RNA genotype 1A standards in plasma which are calibrated against the first WHO HAV 00/560 and parvovirus B19 99/800 International Standards[6,7]. The plasma matrix in which the run control is diluted is manufactured from plasma units that tested negative for all relevant markers of blood borne viruses including HAV-RNA and parvovirus B19 DNA. A reactive HAV-RNA result and a measured parvovirus B19 load of around 10,000 IU/mL on the run control indicates that the NAT method has been performed with sufficient analytical sensitivity and accuracy. A low or non-reactive result for HAV-RNA or major change in measurement of parvovirus B19 DNA concentration is indicative of reduced sensitivity or accuracy of the NAT system and should trigger investigation of the technical performance of the assay. The run control generates concentration values (IU/mL) for parvovirus B19 DNA in the cobas DPX and Procleix Parvo/HAV assays. For HAV-RNA the run control generates sample to cut-off (S/CO) ratios in the Procleix Parvo/HAV TMA assay and Ct values in the cobas DPX real-time PCR assay. Statistical analysis of these assay response values generated over a certain period allows for comparison of analytical performance of NAT reagent lots and laboratory instruments. For this purpose, users of the cobas DPX or Procleix Parvo/HAV assays in combination with the P0266 ViraQ Parvo B19/HAV Check Control can generate a statistical report for post-market performance follow-up[8] using the DataQ Analytics system accessible via the website www.bioqcontrol.com.

Table 2. Detection limits on P0136 HAV genotype 1A standard and WHO parvovirus B19 and HAV standard dilution panels in Grifols Procleix and Roche cobas DPX assays[9].

Standard	Reference panel	NAT method	n	50% LOD (CI) IU/mL	95% LOD (CI) IU/mL
S0036 HAV-RNA genotype 1A standard	P0136 HAV genotype 1A standard dilution panel	Procleix Tigris	7	0.19 (0.09-0.39)	1.42 (0.68-3.10)
1st WHO HAV standard 00/560	-	Procleix Tigris	180	0.20 (0.17-0.22)	0.93 (0.78-1.16)
		Procleix Panther	180	0.24 (0.21-0.27)	1.06 (0.90-1.30)
		Cobas DPX	189	-	1.05 (0.91-1.29)
2nd WHO parvovirus B19 standard 99/802	-	Cobas DPX	189	2.46 (2.04-2.86)	13.9 (11.7-17.4)

Traceability to viral nucleic acid copies and International Units

P0266 ViraQ Parvo B19/HAV Check is prepared from a high titre parvovirus B19 DNA plasma unit (S0037 standard) and a HAV-RNA HM175/18F tissue culture strain spiked in plasma (S0036), both further diluted in plasma negative for other blood borne viruses to reach a concentration of 10 IU/mL for HAV-RNA and 10,000 IU/mL for parvovirus B19 DNA[9]. The S0036 and S0037 standards are directly traceable to the 1st WHO International Standards: 00/560 for HAV-RNA and 99/800 for parvovirus B19V-DNA.

HAV-RNA calibration

The S0036 HAV-RNA standard was calibrated against the 1st WHO 00/560 HAV standard based on the WHO collaborative study[6] results and quantitative measurements performed by one participating laboratory using the Roche LightCycler assay (Sanquin National Screening Service, Amsterdam, The Netherlands) (Table 3). Since there was large variation in the estimated "NAT detectable units" calculated from the qualitative PCR results of the other laboratories, the quantification by the Roche LightCycler assay was considered more reliable than the overall collaborative study results (showing a significant difference between the 1st and 2nd WHO HAV standard knowing to be equivalent)[9]. The final concentration in the S0036 standard was assigned at 5,000,000 IU/mL based on the quantitative Light Cycler results, rounded from an estimate of 53,703 × 100 = 5,370,300 IU/mL (Table 3).

The HAV-RNA quantification in copies/ml is derived from analytical sensitivity studies showing a 63% limit of detection (LOD) of approximately 0.24-0.28 IU/mL in the Procleix assays[9]. According to Poisson distribution, the 63% LOD corresponds to one detectable HAV-RNA copy per assay input volume (0.5 mL). With an estimated 80% detection efficiency, this yields a conversion factor of approximately 8.8-10.6 copies per IU. The P0266 ViraQ Parvo B19/HAV Check Control contains 10 IU/mL of HAV-RNA, corresponding to approximately 100 HAV-RNA copies or virions. This concentration is positioned 7-11 times above the 95% LOD of both Grifols Procleix and Roche cobas DPX assays (Table 2).

Table 3. HAV standard calibration results in WHO collaborative study and in one participating laboratory using the Roche LightCycler Assay[9].

HAV standard	Overall WHO collaborative study estimate IU/mL (95% CI)	Roche LightCycler assay estimate# copies/mL	Sanquin Estimate IU/mL
1st WHO 00/560	100,000 (reference)	416,896	100,000
2nd WHO 00/562	64,565 (41,687-100,000)	426,580	102,329
S0036 1:100	43,652 (23,442-81,283)	223,872	53,703

Geometric mean values on three 10-fold dilutions tested in quadruplicate by Sanquin

Parvovirus B19 DNA calibration

The S0037 parvovirus B19 standard was calibrated against the 1st WHO 99/800 standard through WHO collaborative study data[7] and by quantification in the Roche LightCycler assay by measurements of one participating laboratory in the WHO study (Sanquin National Screening Service, Amsterdam, The Netherlands)[9]. The S0037 standard concentration was calibrated from the results in the LightCycler test at 8.6 (7.5 – 9.9) × 10^{11} IU/mL using a parallel line assay. This result was comparable to the overall estimate of the WHO collaborative study (Table 4).

The conversion from IU to DNA copies is estimated from the 63% LOD of 3.49 IU/mL observed in the Roche cobas DPX assay. Assuming 50-100% PCR detection efficiency with 1 mL input volume, this corresponds to 1-2 copies/mL, yielding a conversion factor of approximately 0.28-0.57 copies per IU. The P0266 ViraQ parvo B19/HAV Check Control contains 10,000 IU/mL or approximately 3,000 Parvo B19-DNA copies/virions.

Table 4. Parvovirus B19 standard calibration results in WHO collaborative study[7] and in one participating laboratory using the Roche LightCycler Assay[9].

Parvovirus B19 standard	WHO collaborative study estimate IU/mL	Roche Light Cycler estimate# IU/mL (95% CI)
1st WHO 99/800	1.0E6 (reference)	1.0E6 (reference)
2nd WHO 99/802	9.12E5	-
S0037 1:10,000	8.71E7	8.62 E7 (7.52E7 – 9.87E7)

using parallel line assay on quadruplicate test results on four 10-fold dilutions of the standards

Traceability chain

The BioQControl S0036 HAV and S0037 parvovirus B19 standards maintain direct traceability to the 1st WHO standards (00/560 and 99/800 respectively, Figures 1 and 2). It must be emphasized that traceability of the S0036 and S0037 standards is no longer guaranteed for the 3rd WHO replacement standards due to different source materials and viral genotype/strains. Altered nucleic acid sequences near primer/probe regions in the 3rd WHO 12/208 parvovirus B19 standard caused significant shifts in the quantitative results of the Roche DPX assay[10] and with introduction of the Grifols Procleix Parvo/HAV Assay on the Panther platform which was adapted to the 3rd WHO 12/208 standard[9].

Figure 1. Traceability chain of P0266 ViraQ Parvo B19/HAV Check Control to the 1st WHO 00/560 HAV standard.

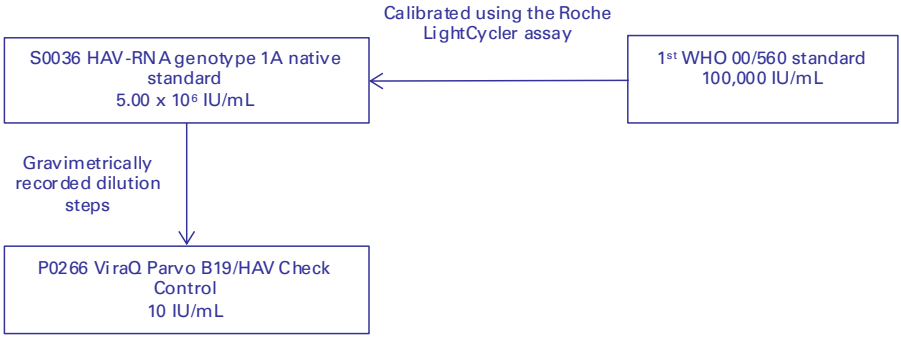
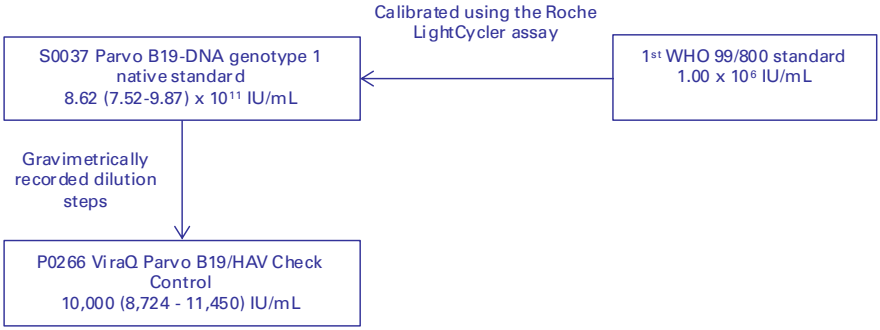


Figure 2. Traceability chain of P0266 ViraQ Parvo B19/HAV Check Control to the 1st WHO 99/800 parvovirus B19 standard.



The manufacturing and quality control procedures ensure consistent virus concentrations across consecutive ViraQ Check Control batches, with sufficient standard stock available to maintain batch-to-batch consistency over a prolonged period[9].

Stability of Parvovirus B19 DNA and HAV-RNA standards and run control

The long-term stability of liquid frozen viral RNA standards stored below –65 °C has been firmly established[6]. Real time stability experiments using an inhouse developed real-time PCR assay for detection of parvovirus B19 DNA and HAV-RNA (Sanquin NSS, Amsterdam, The Netherlands)[5] showed that the S0036 HAV-RNA and S0037 parvovirus B19 DNA standards in the P0266 run control were completely stable for 4.5 years when stored at –30 °C[1,6]. Thus, it can be guaranteed that the run control generates a reactivity rate greater than 99.9% when stored at –30 °C and used before the expiration date (set at four years after preparation of the run control batch).

Biological safety of run control

The virus standards in the P0266 ViraQ Parvo B19/HAV Control containing 10 IU/mL of HAV-RNA and 10,000 IU/mL of parvovirus B19 DNA have not been inactivated. Heat treatment is known to affect the particle integrity of parvovirus B19[12] and the commutability of standards as well as their traceability to the first non-inactivated WHO

International Standards[13]. The infectivity-risk analysis summarized below justifies that inactivation of the virus standards is not necessary for the diagnostic use of the P0266 run control in blood screening laboratories. For this risk analysis the concentrations of virions in the run control and the minimum infectious dose are relevant.

HAV transmission risk assessment

Assuming 1 IU ≈ 10 virions for HAV the P0266 ViraQ Parvo B19/HAV Control contains approximately 100 HAV virions. The minimum infectious dose of HAV via the intravenous route was estimated to be 10-100 virus particles according to Purcell and colleagues[14], but the infectivity was found to be $10^{4.5}$-fold lower in tamarins and chimpanzees when HAV was administered via the faecal-oral route[15]. When the highly attenuated tissue culture adapted HAV strain HM-175 (used for preparation of the run control) was administered orally to human volunteers, doses as high as 10^7 $TCID_{50}$ were not infectious, whereas doses of 10^5 $TCID_{50}$ were not infectious via the intramuscular route[15]. The infectivity of HAV is expected to be further reduced by neutralizing anti-HAV antibodies that are likely present in the plasma pools used for preparation of the run control. Therefore, even though the S0036 HAV standard has not been inactivated, the run control can be safely used by trained laboratory workers who take the necessary precautions.

Parvovirus B19 transmission risk assessment

For safety assessment, a conversion factor of approximately 1 IU ≈ 0.3 copies was used, meaning the P0266 ViraQ Parvo B19/HAV Control contains 10,000 IU/mL or approximately 3,000 parvovirus B19 DNA copies/virions[1,9]. Transmission cases with clinical disease were documented in recipients of clotting factor preparations and SD inactivated pooled plasma contaminated with high viral loads of B19V[1,16,17]. The FDA's Blood Products Advisory Committee reviewed these transmission cases and found evidence of transmission in 18 of 19 recipients of pools containing $10^{7.5}$ to $10^{8.5}$ genome equivalents (geq)/mL whereas 58 recipients of pools containing $\leq 10^{3.5}$ geq/mL remained seronegative[2,17]. This was the basis for the FDA to recommend plasma manufacturers to perform B19V NAT minipool testing and exclude plasma donations with high viral load to ensure that manufacturing pools contain less than 10^4 IU/mL. The reason that plasma pools below 10,000 IU/mL of B19V have not been associated with transmission is the presence of anti-B19V antibodies in the pools since 50-70% of donors have detectable neutralizing anti-B19V antibodies from past infection. Parvovirus B19 transmission has also been well documented in recipients of blood components[18-20]. A German study showed that red blood cells (RBCs) from donors with B19V levels $<10^5$ IU/mL were not associated with B19V transmission in 16 susceptible recipients, but evidence of transmission was found in 9 of 18 RBC transfusions from donors with B19V load greater than 10^5 IU/mL[20]. This finding was in line with two other linked donor-recipient studies in which donor transmitter B19V levels were found to be higher than 10^6 IU/mL[18,19]. In conclusion, this review and risk analysis indicates that the P0266 run control containing 10,000 IU/mL can be considered safe for laboratory workers performing parvovirus B19V NAT donor screening. Heat-inactivation affects the particle integrity of B19V in standards and controls[12] and the impact of chemical inactivation methods is not known. The data reviewed above indicates that the benefit of inactivation of B19V for the safety of laboratory workers is minimal and does not outweigh the risk of damaging the virus particles (and with that the commutability and stability of the standard).

Kit Contents (materials provided)

The run control contains human plasma without preservatives and is provided in two formats in each platform as detailed in Table 5.

Table 5. Description of P0266 ViraQ Parvo B19/HAV Check# kit formats and contents.

Platform	Cat. Code	GTIN/UDI-DI code^	Quantity (10 mL tubes)	Sample volume	Secondary Packaging
cobas®	P0266/01	8718719830925	60 tubes	1.5 mL (+overfill)	Rack in box
	P0266/02	8718719832202	10 tubes	1.5 mL (+overfill)	Plastic zip bag
Procleix®	P0266/03	8718719830536	60 tubes	1.4 mL (+overfill)	Rack in box
	P0266/04	8718719830567	10 tubes	1.4 mL (+overfill)	Plastic zip bag

^ Global Trade Item Number = Unique Device Identification - Device Identifier (UDI-DI) code,
# Global Model Number (GMN) = Basic UDI-DI code for P0266 is 871871983P0266F3

To facilitate automation the run control is presented in a polypropylene tube with screw cap comparable in size to vacutainer tubes used for donor sample collection. In addition, the label includes a barcode identifying the product, sequential batch number, and markers. The barcode of each run control tube of the P0266/01 and P0266/02 product variants is unique and can be read by the automated cobas systems, whereas the barcodes on the P0266/03 and /04 variants are suitable for the Panther instruments.

Materials required but not provided

The test kits and liquid handling devices by the NAT manufacturer as specified in Table 1, a water bath of 37 °C for quickly thawing of run control and a vortex instrument for thorough mixing of samples are not provided.

Storage instructions

The run controls should be stored at or below –30 °C for a maximum of four years after preparation of the run control batch and not be used after the expiry date[9]. Once thawed the run control samples should be used within 8 hours. During this period, when the run control is not in use, store sample at 2-8 °C. Do not refreeze the controls after thawing to prevent formation of cryoprecipitates. Any control sample that appears cloudy or contains precipitates after thawing and mixing should be discarded.

Warning and precautions

Although P0266 ViraQ Parvo B19/HAV Check control contains native viral particles at concentrations considered safe for laboratory workers[9] the plasma may still be potentially bio-hazardous. The matrix is prepared from human blood plasma that tested negative for blood borne viruses (HBV-DNA, HCV-RNA, HIV-RNA, HBsAg, anti-HBc, anti-HIV, anti-HCV and anti-*Treponema pallidum*). No test method can offer complete assurance that products derived from human blood cannot transmit (unknown) infectious agents. The run control should only be used by trained laboratory workers who are aware of the potential risk of infectious agents in human plasma samples and take the necessary precautions. Observe the universal precautions for prevention of transmission of infectious agents when handling these materials[21,22]. In case of a serious incident related to the use of the run control, report it immediately to BioQControl's technical support group (tel: +31 (0)72 2020 730; e-mail: info@bioqcontrol.com) and to the competent authority of the country/EU Member State where the incident occurred.

- 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 run controls are handled.

- Disinfect spills using a 0.5% hypochlorite solution (1:10 v/v household bleach) or equivalent disinfectant.
- Dispose unused or spilled materials according to the normal practices for biological waste disposal in your institution.
- If precipitates are visible, mix the run controls for 2 minutes thoroughly.
- Once thawed, do not re-freeze and thaw the run control samples to avoid formation of cryoprecipitates that could alter reactivity or cause pipetting errors in the automated sampling systems.
- Store run controls in an upright position.

Reagent preparation

- Thaw the run control quickly in a water bath at 37 °C.
- Mix gently during thawing until contents are just thawed.
- Immediately after ice clot has disappeared remove the run control tube from the water bath.
- Before testing allow the run control tube to adapt to room temperature.
- Mix the run control tube thoroughly prior to use with a vortex instrument.
- Give a short spin in a centrifuge to remove liquid before releasing screw cap from vial.
- Minimise the time from thawing until testing of the control samples.
- Test run control sample within 8 hours after thawing.
- After thawing when not in use, store run control tube at 2-8 °C.

Performance Characteristics

cobas DPX assay

The cobas DPX assay gives qualitative results (reactive, nonreactive, or invalid) for both parvovirus B19 DNA and HAV-RNA. If reactive, an IU/mL value is generated for Parvo B19-DNA and a Cycle threshold (Ct) value is generated for HAV-RNA, together with an internal cobas DPX control. P0266 ViraQ Parvo B19/HAV Check Control (10,000 IU/mL parvovirus B19 DNA and 10 IU/mL HAV-RNA) is expected to be reactive in more than 99.9% of cobas DPX test runs.

For parvovirus B19 DNA, IU/mL results follow a lognormal distribution and can be log-transformed for potency calculations and trend analysis. For HAV-RNA, Ct values typically follow a normal distribution and can be used untransformed in Levey–Jennings QC charts for performance monitoring.

Procleix Parvo/HAV assay

The Procleix Parvo/HAV assay gives qualitative results (reactive, nonreactive, or invalid) for both parvovirus B19 DNA and HAV-RNA. If reactive, a quantitative IU/mL value is generated for parvovirus B19 DNA and a sample-to-cut-off (S/CO) ratio is generated for HAV-RNA. P0266 ViraQ Parvo B19/HAV Check Control is expected to be reactive in more than 99.9% of TMA test runs.

For parvovirus B19 DNA, IU/mL results follow a lognormal distribution and should be log-transformed for potency calculations and trend analysis. For HAV-RNA, S/CO ratios follow a non-parametric distribution and are not suitable for potency calculations, but they can be monitored for QC purposes.

Expected assay response values on run control

The expected results for the P0266 ViraQ Parvo B19/HAV Check Control are presented in Table 6 for the cobas DPX assay and in Table 7 for the Procleix Parvo/HAV assay.

Table 6. Expected results on P0266 ViraQ Parvo B19/HAV Check Control in the cobas DPX assay.

Parvo B19-DNA IU/mL values range	HAV-RNA Ct values range
7,918 – 17,962	34.7 – 36.8

Table 7. Expected results on P0266 ViraQ Parvo B19/HAV Check Control in the Procleix Parvo/HAV assay.

Parvo B19-DNA IU/mL values range	HAV-RNA S/CO values range
3,926 – 14,899	2.6 – 3.4

Each assay reagent lot appears to have its own dose response line and distribution of response values on P0266 ViraQ Parvo B19/HAV Check Control. This depends on the analytical sensitivity and accuracy of the assay reagent lots that are in use. Thus, it cannot be guaranteed that the assay response values will always fall within these ranges. P0266 ViraQ Parvo B19/HAV run control serves as an independent standard for monitoring the reproducibility of the cobas DPX and Procleix Parvo/HAV test runs and reagent lots over time.

Interpretation of Results

The run control should be tested in a manner identical to that of clinical specimens/plasma pools and the result be calculated according to the instructions for use of the NAT procedure. Subsequent test runs can be analysed by appropriate statistical approaches on the response values obtained on the external control samples. A software system (DataQ Analytics) is available via the website www.bioqcontrol.com for entering response values and generating a statistical report with assay-specific calculations for preparing a Levey-Jennings Chart.

cobas DPX assay – parvovirus B19 DNA

Calculations

The log-transformed IU/mL results on parvovirus B19 DNA can be used for potency calculations and trend analysis. Use Student-t-values belonging to the 95% and 99% predictive intervals (Table 8). Calculate the 95% and 99% predictive limits as follows:

- 99% Lower limit: Average log (IU/mL) – 99% t-Value x SD log (IU/mL) x $\sqrt{(1 + 1/n)}$
- 95% Lower limit: Average log (IU/mL) – 95% t-Value x SD log (IU/mL) x $\sqrt{(1 + 1/n)}$
- 99% Upper limit: Average log (IU/mL) + 99% t-Value x SD log (IU/mL) x $\sqrt{(1 + 1/n)}$
- 95% Upper limit: Average log (IU/mL) + 95% t-Value x SD log (IU/mL) x $\sqrt{(1 + 1/n)}$

Table 8. Student t value to calculate predictive interval for 30 test runs.

Runs (n)	t-value at 95% int.	t-value at 99% int.
30	2.045	2.756
∞	1.960	2.576

Test the run control at least 30 times during the reference period. The predictive intervals in IU/mL can be calculated by back-transforming the log predictive limits. The Levey-

Jennings chart (Figure 3) is designed to identify individual aberrant values outside the 95% and 99% predictive intervals. By collecting additional data, the chart characteristics may be updated.

Use Nelson rules[23] to identify deviations in the Levey-Jennings trend analysis.

The cobas DPX Parvo B19-DNA test results on P0266 ViraQ Parvo B19/HAV Check control are expected to be reactive in 100% of cases with IU/mL values falling within the ranges given in Table 6.

Levey-Jennings Chart

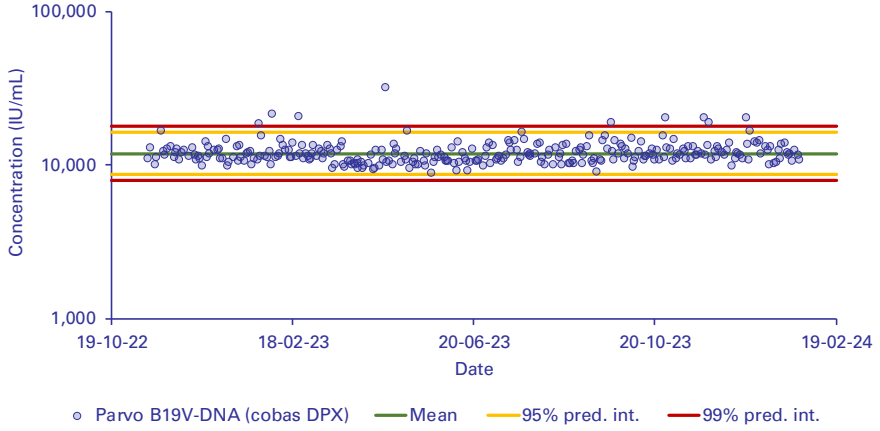
The IU/mL values obtained in consecutive cobas DPX test runs for parvovirus B19 DNA can be plotted in a Levey-Jennings chart using the statistical report function of the DataQ Analytics system available on www.bioqcontrol.com as shown in the similar chart of Figure 3.

Table 9 summarizes the Levey-Jennings chart parameters in Figure 3, which were deduced from the IU/mL values reported on one P0266 ViraQ Parvo B19/HAV batch (B4261-004) by one laboratory over a period of one year.

Table 9. Geometric mean IU/mL values with 95% and 99% predictive intervals as found on one batch of P0266 ViraQ Parvo B19/HAV Check Control in 302 cobas DPX test runs in the period from 12-11-2022 to 25-01-2024 (data in Figure 3).

Assay	n	Mean IU/mL	95% pred. int. Ct	99% pred. int. Ct
cobas DPX	302	11,925	8,738 – 16,274	7,918 – 17,961

Figure 3. Levey-Jennings QC chart of cobas DPX assay IU/mL values for parvovirus B19 DNA on one batch of P0266 ViraQ Parvo B19/HAV Check Control.



Post-market performance follow-up of run control batches

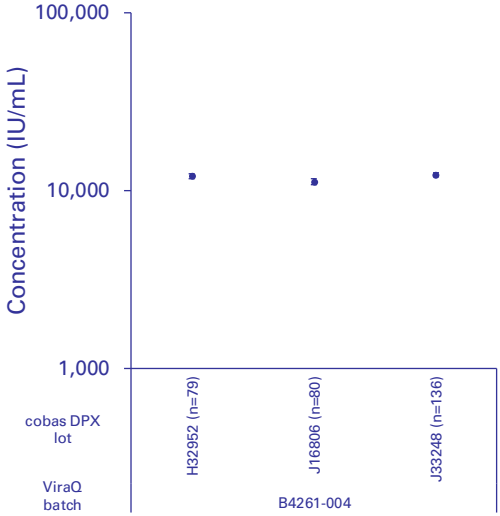
One can use P0266 ViraQ Parvo B19/HAV Check in combination with the DataQ Analytics system on www.bioqcontrol.com to compare the IU/mL values on different run control batches, cobas DPX reagent lots or cobas 5800/6800/8800 instruments. Table 10 compares the variation in mean IU/mL values on different cobas DPX reagent lots for parvovirus B19

DNA. There is minimal variation in IU/mL values on consecutive cobas DPX reagent lots as is visualized in Figure 4 showing the parvovirus B19 DNA results over a period of one year (same data as in Table 10).

Table 10. Comparison of geometric mean Parvo B19-DNA IU/mL values (and 95% confidence interval (CI)) on one P0266 ViraQ Parvo B19/HAV Check Control batch and between consecutive cobas DPX reagent lots.

P0266 ViraQ run control batch	cobas reagent lot	n	Mean concentration (IU/mL; 95% CI)
B4261-004	*H31217*	*7*	*12,119 (10,467 - 14,032)*
	H32952	79	12,094 (11,713 - 12,486)
	J16806	80	11,179 (10,766 - 11,607)
	J33248	136	12,277 (11,969 - 12,593)
	All	**302**	**11,925 (11,714 - 12,140)**
All	**All**	**302**	**11,925 (11,714 - 12,140)**

Figure 4. Geometric mean IU/mL (and 95% CI) values for parvovirus B19 DNA in cobas DPX assay reagent lots on one P0266 ViraQ Parvo B19/HAV Check run control batch (data in Table 10).



cobas DPX assay – HAV-RNA

Calculations

Use Student-t-values belonging to the 95% and 99% predictive intervals (Table 8). Calculate the 95% and 99% predictive limits as follows:

- 99% Lower limit: Average – 99% Student-t-Value x SD x $\sqrt{(1 + 1/n)}$
- 95% Lower limit: Average – 95% Student-t-Value x SD x $\sqrt{(1 + 1/n)}$
- 99% Upper limit: Average + 99% Student-t-Value x SD x $\sqrt{(1 + 1/n)}$
- 95% Upper limit: Average + 95% Student-t-Value x SD x $\sqrt{(1 + 1/n)}$

Test the run control at least 30 times during the reference period. Since Ct values are used no log transformation is required and predictive intervals can be calculated from the

arithmetic mean and standard deviation (SD). The Levey-Jennings chart (Figure 5) is designed to identify individual aberrant values outside the 95% and 99% predictive intervals. By collecting additional data, the chart characteristics may be updated.

Use Nelson rules[23] to identify deviations in the Levey-Jennings trend analysis.

The cobas DPX HAV-RNA test results on P0266 ViraQ Parvo B19/HAV Check control are expected to be reactive in 99.5-100% of cases according to probit analysis on the 1st WHO HAV standard 00/560 dilution panel (Table 2). Repeatedly non-reactive results are indicative of a significantly reduced analytical sensitivity of the NAT system. A single event of a non-reactive result is however possible without deterioration of the test system and can be explained by Poisson distribution.

Levey-Jennings Chart

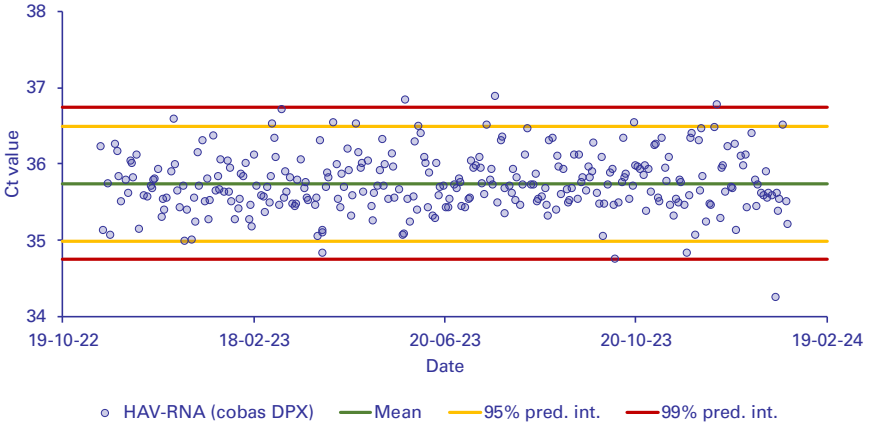
The Ct values obtained in consecutive cobas DPX test runs for HAV-RNA can be plotted in a Levey-Jennings chart using the statistical report function of the DataQ Analytics system available on www.bioqcontrol.com as shown in the similar chart of Figure 5.

Table 11 summarizes the Levey-Jennings chart parameters in Figure 5, which were deduced from the Ct values reported on one P0266 ViraQ Parvo B19/HAV batch (B4261-004) by one laboratory over a period of one year.

Table 11. Average Ct values with 95% and 99% predictive intervals as found on one batch of P0266 ViraQ Parvo B19/HAV Check Control in 300 cobas DPX test runs in the period from 12-11-2022 to 25-01-2024 (data in Figure 5).

Assay	n	Average Ct	95% pred. int. Ct	99% pred. int. Ct
cobas DPX	300	35.74	34.99 – 36.50	34.75 – 36.74

Figure 5. Levey-Jennings QC chart of cobas DPX assay Ct values for HAV-RNA on one batch of P0266 ViraQ Parvo B19/HAV Check Control.



Post-market performance follow-up of run control batches

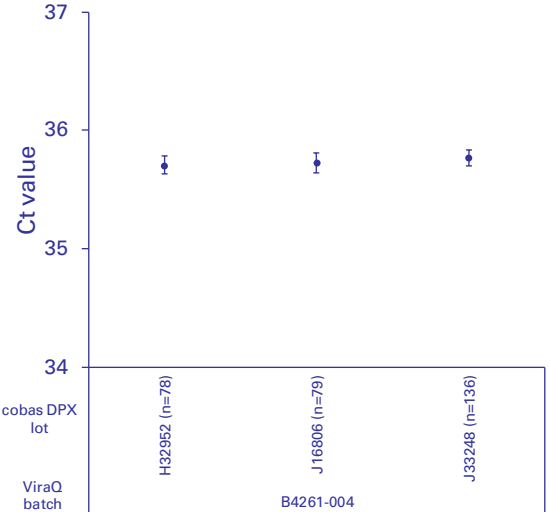
One can use P0266 ViraQ Parvo B19/HAV Check in combination with the DataQ Analytics system on www.bioqcontrol.com to compare the Ct values on different run control

batches, cobas DPX reagent lots or cobas 5800/6800/8800 instruments. Table 12 compares the variation in mean Ct values on different cobas DPX reagent lots for HAV-RNA. There is minimal variation in Ct values on consecutive cobas DPX reagent lots as is visualized in Figure 6 showing the HAV-RNA results over a period of one year (same data as in Table 12).

Table 12. Comparison of mean HAV-RNA Ct values (and 95% confidence interval (CI)) on one P0266 ViraQ Parvo B19/HAV Check Control batch and between consecutive cobas DPX reagent lots.

P0266 ViraQ run control batch	cobas reagent lot	n	Average Ct (95% CI)
B4261-004	*H31217*	*7*	*35.77 (35.31 - 36.24)*
	H32952	78	35.71 (35.63 - 35.79)
	J16806	79	35.73 (35.64 - 35.81)
	J33248	136	35.77 (35.70 - 35.84)
	All	**300**	**35.74 (35.70 - 35.79)**
All	**All**	**300**	**35.74 (35.70 - 35.79)**

Figure 6. Average Ct (and 95% CI) values for HAV-RNA in cobas DPX assay reagent lots on one P0266 ViraQ Parvo B19/HAV Check run control batch (data in Table 12).



Procleix Parvo/HAV assay – parvovirus B19 DNA

Calculations

The log-transformed IU/mL results on Parvo-B19 DNA can be used for potency calculations and trend analysis. Use Student-t-values belonging to the 95% and 99% predictive intervals (Table 8). Calculate the 95% and 99% predictive limits as follows:

- 99% Lower limit: Average log (IU/mL) – 99% t-Value x SD log (IU/mL) x $\sqrt{(1 + 1/n)}$
- 95% Lower limit: Average log (IU/mL) – 95% t-Value x SD log (IU/mL) x $\sqrt{(1 + 1/n)}$
- 99% Upper limit: Average log (IU/mL) + 99% t-Value x SD log (IU/mL) x $\sqrt{(1 + 1/n)}$
- 95% Upper limit: Average log (IU/mL) + 95% t-Value x SD log (IU/mL) x $\sqrt{(1 + 1/n)}$

Test the run control at least 30 times during the reference period. The predictive intervals in IU/mL can be calculated by back-transforming the log predictive limits. The Levey-Jennings chart (Figure 7) is designed to identify individual aberrant values outside the 95% and 99% predictive intervals. By collecting additional data, the chart characteristics may be updated.

Use Nelson rules[23] to identify deviations in the Levey-Jennings trend analysis.

The parvovirus B19 DNA test results on P0266 ViraQ Parvo B19/HAV Check control in the Procleix Parvo/HAV assay are expected to be reactive in 100% of cases with IU/mL values falling within the ranges given in Table 7.

Levey-Jennings Chart

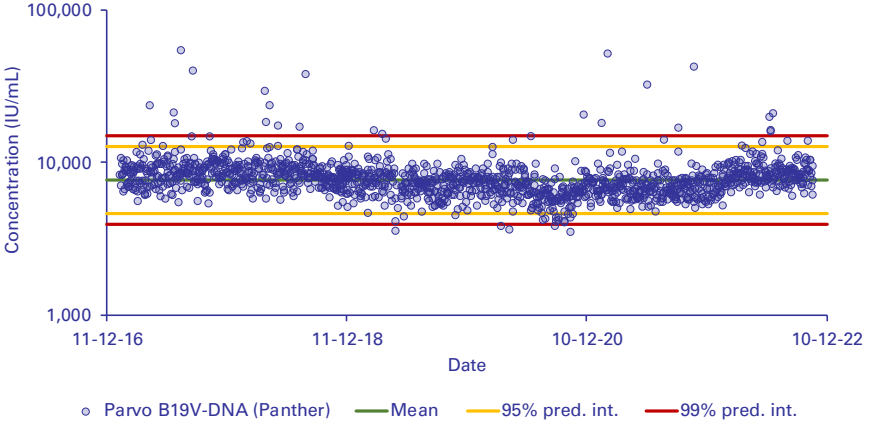
The IU/mL values obtained in consecutive Procleix Parvo/HAV test runs for Parvo B19-DNA can be plotted in a Levey-Jennings chart using the statistical report function of the DataQ Analytics system available on www.bioqcontrol.com as shown in the similar chart of Figure 7.

Table 13 summarizes the Levey-Jennings chart parameters in Figure 7, which were deduced from the IU/mL values reported on three P0266 ViraQ Parvo B19/HAV batches (B4261-001, 002, and 003) by one laboratory over a period of six years.

Table 13. Geometric mean IU/mL values with 95% and 99% predictive intervals as found on three batches of P0266 ViraQ Parvo B19/HAV Check Control in 1473 Procleix Parvo/HAV test runs in the period from 21-01-2017 to 29-10-2022 (data in Figure 7).

Assay	n	Mean IU/mL	95% pred. int. Ct	99% pred. int. Ct
Procleix Parvo/HAV	1473	7,648	4,606 – 12,700	3,926 – 14,899

Figure 7. Levey-Jennings QC chart of Procleix Parvo/HAV assay IU/mL values for Parvo B19-DNA on three batches of P0266 ViraQ Parvo B19/HAV Check Control.



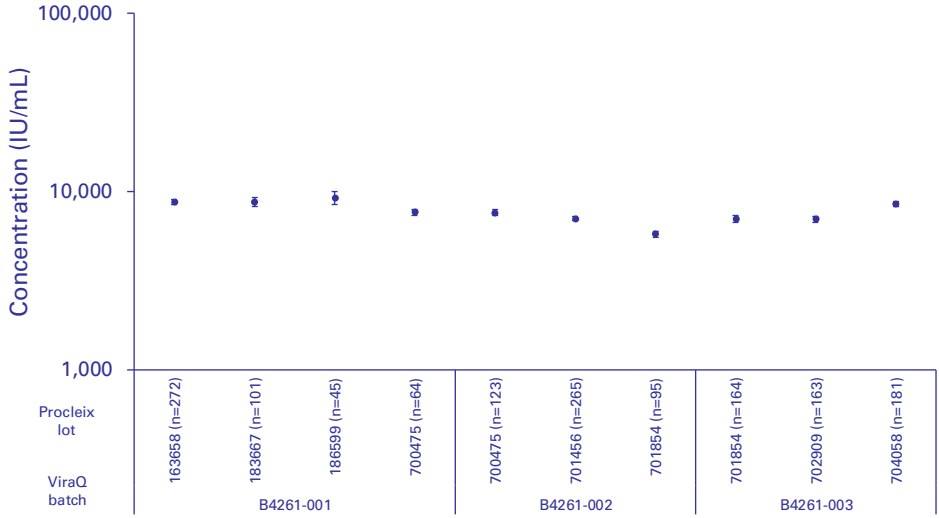
Post-market performance follow-up of run control batches

One can use P0266 ViraQ Parvo B19/HAV Check in combination with the DataQ Analytics system on www.bioqcontrol.com to compare the IU/mL values on different run control batches, Procleix Parvo/HAV reagent lots or Procleix Panther instruments. Table 14 compares the variation in mean IU/mL values on P0266 ViraQ Parvo B19/HAV batches and Procleix Parvo/HAV reagent lots for Parvo B19-DNA. There is some variation in IU/mL values on consecutive Procleix Parvo/HAV reagent lots as is visualized in Figure 8 showing the Parvo B19-DNA results over a period of six years (same data as in Table 14).

Table 14. Comparison of geometric mean parvovirus B19 DNA IU/mL values (and 95% confidence interval (CI)) on three P0266 ViraQ Parvo B19/HAV Check Control batches and between consecutive Procleix Parvo/HAV reagent lots.

P0266 ViraQ run control batch	Procleix reagent lot	n	Mean concentration (IU/mL; 95% CI)
B4261-001	163658	272	8,707 (8,449 – 8,972)
	183667	101	8,734 (8,284 - 9,209)
	186599	45	9,188 (8,477 - 9,959)
	700475	64	7,652 (7,371 - 7,945)
	All	**482**	**8,608 (8,417 - 8,802)**
B4261-002	700475	123	7,611 (7,312 - 7,923)
	701456	265	7,055 (6,897 - 7,217)
	701854	95	5,763 (5,516 - 6,021)
	All	**483**	**6,912 (6,775 - 7,052)**
B4261-003	701854	164	7,051 (6,757 - 7,358)
	702909	163	6,987 (6,742 - 7,241)
	704058	181	8,542 (8,305 - 8,787)
	All	**508**	**7,528 (7,364 - 7,695)**
All	**All**	**1473**	**7,648 (7,548 - 7,750)**

Figure 8. Geometric mean IU/mL (and 95% CI) values for Parvo B19-DNA in Procleix Parvo/HAV assay reagent lots on three P0266 ViraQ Parvo B19/HAV Check run control batches (data in Table 14).



Procleix Parvo/HAV assay – HAV-RNA

Calculations

Since the Procleix Parvo/HAV assay returns non-parametrically distributed S/CO ratios, predictive ranges are based on the percentiles of the data range. Calculate the 95% and 99% predictive limits as follows:

- 99% Lower limit: (interpolated) 0.005th percentile
- 95% Lower limit: (interpolated) 0.025th percentile
- 99% Upper limit: (interpolated) 0.995th percentile
- 95% Upper limit: (interpolated) 0.975th percentile

Test the run control at least 30 times during the reference period. The Levey-Jennings chart (Figure 9) is designed to identify individual aberrant values outside the 95% and 99% ranges. By collecting additional data, the chart characteristics may be updated.

Use Nelson rules[23] to identify deviations in the Levey-Jennings trend analysis.

The HAV-RNA test results on P0266 ViraQ Parvo B19/HAV Check control in the Procleix Parvo/HAV assay are expected to be reactive in 99.5-100% of cases according to probit analysis on the inactivated S0036 HAV-RNA genotype 1A standard dilutions in the P0136 reference panel. Repeatedly non-reactive results are indicative of a significantly reduced analytical sensitivity of the NAT system. A single event of a non-reactive result is however possible without deterioration of the test system and can be explained by Poisson distribution.

Levey-Jennings Chart

The S/CO ratios obtained in consecutive Procleix Parvo/HAV test runs for HAV-RNA can be plotted in a Levey-Jennings chart using the statistical report function of the DataQ

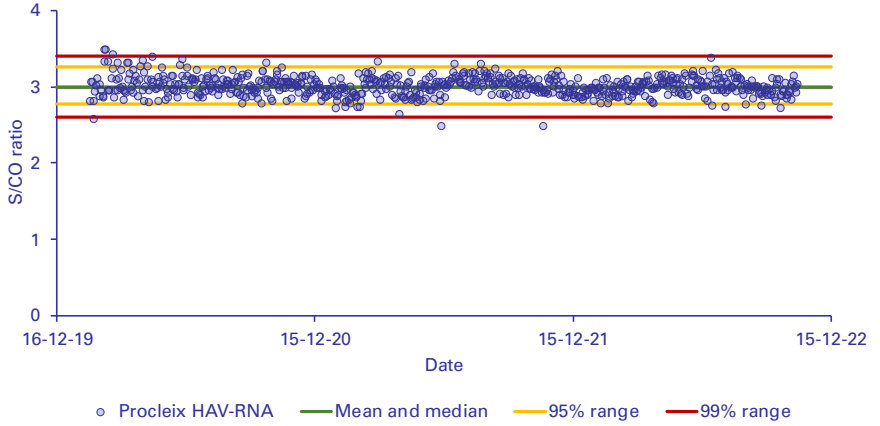
Analytics system available on www.bioqcontrol.com as shown in the similar chart of Figure 9.

Table 15 summarizes the Levey-Jennings chart parameters in Figure 9, which were deduced from the S/CO ratios reported on two P0266 ViraQ Parvo B19/HAV batches (B4261-002 and 003) by one laboratory over a period of 2 years.

Table 15. Median S/CO ratios with 95% and 99% ranges as found on two batches of P0266 ViraQ Parvo B19/HAV Check Control in 699 Procleix Parvo/HAV test runs in the period from 01-02-2020 to 29-10-2022 (data in Figure 9).

Assay	n	Median S/CO	95% range S/CO	99% range S/CO
Procleix Parvo/HAV	699	3.00	2.78 – 3.27	2.61 – 3.40

Figure 9. Levey-Jennings QC chart of Procleix Parvo/HAV assay S/CO ratios for HAV-RNA on two batches of P0266 ViraQ Parvo B19/HAV Check Control.



Post-market performance follow-up of run control batches

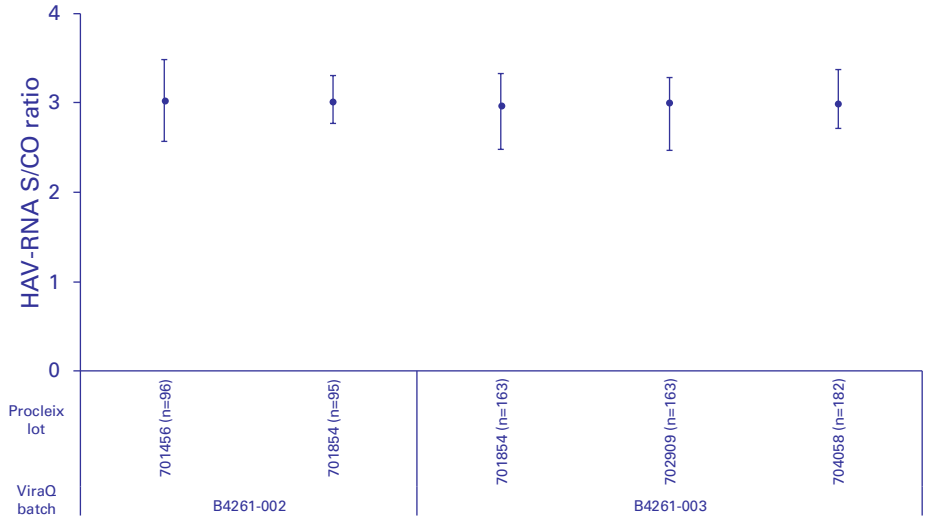
One can use P0266 ViraQ Parvo B19/HAV Check in combination with the DataQ Analytics system on www.bioqcontrol.com to compare the S/CO ratios on different run control batches, Procleix Parvo/HAV reagent lots or Procleix Panther instruments. Table 16 and Figure 10 compare the median S/CO ratios on P0266 ViraQ Parvo B19/HAV batches and Procleix Parvo/HAV reagent lots for HAV-RNA, showing no indications for changes in analytical sensitivity.

The S/CO responses on ViraQ Parvo/HAV Check in the Procleix assay are not normally distributed, since >98% of S/CO ratios are in the saturation range of the assay. Therefore, an increase in S/CO ratios below the saturation range will increase the difference between the median and the average of S/CO values ($\Delta S/CO_{M-A}$). Hence, $\Delta S/CO_{M-A}$ is expected to become higher with lower analytical sensitivity of the NAT system and may be used for trend analysis[2].

Table 16. Comparison of median HAV-RNA S/CO ratios (and min - max range) on P0266 ViraQ Parvo B19/HAV Check Control batches and between consecutive Procleix Parvo/HAV reagent lots.

P0266 ViraQ run control batch	Procleix reagent lot	n	Median S/CO (range)
B4261-002	701456	96	3.03 (2.57 - 3.48)
	701854	95	3.01 (2.77 - 3.31)
	All	**191**	**3.02 (2.57 - 3.48)**
B4261-003	701854	163	2.97 (2.48 - 3.33)
	702909	163	3.00 (2.47 - 3.29)
	704058	182	2.99 (2.71 - 3.37)
	All	**508**	**2.99 (2.47 - 3.37)**
All	**All**	**699**	**3.00 (2.47 - 3.48)**

Figure 10. Median S/CO (and min – max range) ratios for HAV-RNA in Procleix Parvo/HAV assay reagent lots on P0266 ViraQ Parvo B19/HAV Check run control batches (data in Table 16).



Limitations

- P0266 ViraQ Parvo B19/HAV Check Control cannot be used to evaluate the analytical or diagnostic sensitivity of NAT blood screening assays (although a significant reduction of analytical sensitivity of the NAT system can become apparent with repeated occurrence of non-reactive results and weak reactive results for HAV-RNA).
- The concentration of 10,000 IU/mL of parvovirus B19 in the P0266 ViraQ Parvo B19/HAV Check Control is directly traceable to the first WHO 99/800 standard and indirectly also to the 2nd WHO99/802 standard but not to the 3rd WHO 12/208 replacement standard due to altered nucleic acid sequence near the primer/probe regions of the NAT systems[9,10].
- P0266 ViraQ Parvo B19/HAV Check Control must not be substituted for the mandatory controls or calibrators provided with NAT test kits for calculating the cut-off and/or criteria for releasing test results.

- The Poisson distribution in samples with low HAV-RNA concentrations cannot guarantee that 100% reactive results will always be found on P0266 ViraQ Parvo B19/HAV Check Control in NAT blood screening assays. Therefore, the response values on the run controls should not be used for a decision to accept or reject the test run.
- The expected ranges of results in the cobas and Procleix assays on P0266 ViraQ Parvo B19/HAV Check Control that are presented in this package insert were based on performance evaluation studies involving a limited number of run control batches and NAT reagent lots. It cannot be guaranteed that slightly different results will not be found on other run control batches, assay reagent lots or instruments.
- It cannot be excluded that some variation exists between the performance of consecutive batches of the P0266 ViraQ Parvo B19/HAV Check control due to matrix effects or other manufacturing variables.

References

1. Blümel J, Burger R, Drosten C et al (Arbeitskreis Blut). Parvovirus revised. Transfus Med Hemother 2010;37:339-350
2. Food and Drug Administration (FDA) – Blood Products Advisory Committee: Nucleic acid testing of blood donors for human parvovirus B19. September 1999. www.fda.gov/ohrms/dockets/ac/99/transcpt/3548t1.rtf.
3. Koppelman M, Cuypers T, Emrich T and Zaaijer H, Quantitative real-time detection of parvovirus B19 DNA in plasma Transfusion 2004;44:97-103.
4. Koppelman MH, Van Swieten P, Cuijpers HT. Real-time polymerase chain reaction detection of parvovirus B19 DNA in blood donations using a commercial and an in-house assay. Transfusion 2011;51:1346-54.
5. Molenaar-de Backer MW, de Waal M, Sjerps MC, Koppelman MHGM. Validation of new real-time polymerase chain reaction assays for detection of hepatitis A virus RNA and parvovirus DNA. Transfusion 2016;56:440-8.
6. Saldanha J, Heath A, Lelie PN, Pisani G, Yu MY and the Collaborative Study Group. A World Health Organisation (WHO) International Standard for Hepatitis A virus (HAV) Nucleic Acid Amplification Technology (NAT) Assays. Vox Sanguinis 2005:89:52-8
7. Saldanha J, Lelie PN, Yu MW. Heath A and the B19 Collaborative Study Group. Establishment of the first World Health Organization International Standard for human parvovirus B19 DNA nucleic acid amplification techniques. Vox Sang. 2002;82:24-31
8. Kerst S, Lelie PN. Post-market performance follow-up report of P0266 ViraQ Parvo B19/HAV Check. VR4121. Technical Documentation BioQControl.
9. Lelie PN, Van Drimmelen AAJ, Kerst S. Design and performance verification of P0266 ViraQ Parvo B19/HAV Check Control. VR4064. Technical documentation BioQControl.
10. Pisani G, Cristiano K, Fabi S, Simeoni M, Gaggliolo A. A significantly lower potency observed for the 3rd WHO International Standard for parvovirus B19V-DNA with the cobas TaqScreen DPX test. Vox Sanguinis 2016:111;115-119
11. Van Drimmelen AAJ, Lelie PN. Stability of ViraQ run controls for HBV/HCV/HIV-NAT. VR4058. Technical documentation BioQControl
12. Blümel J, Schmidt I, Willkommen H, Löwer J. Inactivation of parvovirus B19 during pasteurization of human serum albumin. Transfusion 2002;42:1011-1018
13. Lelie N, Van Drimmelen H. Accuracy of quantitative HIV-1 RNA test methods at 1000 copies/mL and the potential impact of differences in assay calibration on therapy monitoring of patients. J Med Virology 2020; 92:3246-3253

14. Purcell RH, Feinstone SM, Tice hurst JR, et al. Hepatitis A virus. In Vyas GN, Dienstag JL, Hoofnagle JH, editors Viral hepatitis and liver disease. Orlando (FL): Grune & Straton:1984. p. 9-22
15. Purcell RH, Wong DC, Shapiro M, Relative Infectivity of Hepatitis A Virus by the Oral and Intravenous Routes in 2 Species of Nonhuman Primates. The Journal of Infectious Diseases 2002;185:1668–71
16. Koenigbauer UF, Eastlund T, Day JW, Clinical illness due to parvovirus B19 infection after infusion of solvent/detergent pooled plasma. Transfusion 2000;40:1203-1206
17. Brown KE, Young NS, Alving BM, Barbosa LH. Parvovirus B19; implications for transfusion medicine. Summary of a workshop. Transfusion 2001;41:130-135
18. Yu M-YW, Alter HJ, Luisa MA. Theimer V, Geng Y, Schechterly S, Colvin CA, Luban NLC. Parvovirus B19 infection transmitted by transfusion of red blood cells confirmed by molecular analysis of linked donor and recipient samples. Transfusion 2010;50:1712-1721
19. Kleinman SH, Glynn SA, Lee TH, Tobler LH, Schlumpf KS, Todd DS, Qiao H, Yu MY, Busch MP; National Heart, Lung and Blood Institute Retrovirus Epidemiology Donor Study II (NHLBI REDS-II). A linked donor-recipient study to evaluate parvovirus B19 transmission by blood component transfusion. Blood 2009;114:3677-83
20. Hourfar MK, Mayer-Wohlfart U, Thermann A, Sireis W, Seifried E, Schrezenmeier H., Schmidt M. Recipients potentially infected with parvovirus B19 by red blood cell products. Transfusion 2011.51:129-36
21. Centers for Disease Control (CDC). Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other blood borne pathogens in health-care settings. MMWR 1988; 37:377-388.
22. 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.
23. Nelson LS, "The Shewhart Control Chart—Tests for Special Causes". Journal of Quality Technology 1984;16, no. 4: 238-239 https://doi.org/10.1080/00224065.1984.11978921

Revision history

Date	Version	Description of change
July 2026	V2.4	Figure 1 updated to align with production optimization. Contact information added for reporting serious incidents in chapter “Warning and precautions”. Revision history table added. Symbol for positive control added.

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