Bio QControl

P0266
ViraQ Parvo B19/HAV Check

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

The P0266 ViraQ Parvo B19-DNA/HAV Check run control is a standardised Parvo B19-DNA/HAV-RNA sample quantified in IU/ml and is intended to be used in blood screening laboratories and diagnostic laboratories to monitor the sensitivity and accuracy of molecular diagnostic test procedures for quantitative detection of Parvo B19 DNA (B19V-DNA) and qualitative detection of HAV RNA in blood samples. This product can be used in conjunction with (real time) PCR and other nucleic acid amplification tests. The results obtained on the samples are suitable for independent monitoring of the (analytical) sensitivity and accuracy of quantification in IU/ml. P0266 ViraQ Parvo B19-DNA/HAV Check must not be used to replace the internal controls of the assay. P0266 ViraQ Parvo B19-DNA/HAV Check is for performance evaluation only (PEO).

Table 1. Test kit covered by this run control

Equipment	Agent	Test kit
TIGRIS®	Hepatitis A virus	Procleix Duplex®
PANTHER®	Parvo B19 virus	Procleix Duplex®

Key to Symbols Used


Manufacturer

Lot number

Catalogue number

Store below -30°C

Biological substance Category B


Device for performance evaluation


Expiry date

Contents


Caution


Read instructions for use

Principle of method

The P0266 ViraQ Parvo B19-DNA/HAV Check run control has been formulated to mimic human plasma specimens containing 10.000 IU/ml parvo-B19-DNA[1,2] and 10 IU/ml of HAV-RNA[3]. The run control contains Parvo B19-DNA genotype 1 and HAV-RNA genotype 1b standards diluted in human plasma. P0266 ViraQ Parvo B19-DNA/HAV Check enables blood screening laboratories to monitor the performance of transcription mediated amplification (TMA) or polymerase chain reaction (PCR) assays for the qualitative detection of hepatitis A virus (HAV)-RNA and quantitative detection of Parvo B19 virus (parvo B19-DNA) in plasma or serum samples. The sample is suitable as a sensitivity control for HAV RNA and as quantitative control for B19V-DNA test runs. The concentration level is chosen at 4 to 5 times the 95 % lower limit of detection (LOD) for HAV-RNA of the Procleix Duplex assay while the Parvo B19-DNA concentration is equal to the concentration cut-off level defined by the Pharmacopeia's for plasma pool testing[4,5,6,7]. The run control generates a quantitative result for parvo B19-DNA and a sample to cut-off (S/CO) ratio for HAV-RNA.
The run control is designed such, that highly concentrated viral stock solutions are diluted in a pool of plasma units that tested negative for viral markers (NAT and serology, including B19V-DNA and HAV-RNA). Since the majority of blood donations do contain naturally acquired antibodies against HAV and Parvo B19, the negative plasma pool designed for the manufacturing of the run control will contain these antibodies and will cause a natural infectivity neutralisation. However, this does not result in full non-infectious run control. The neutralized HAV and Parvo B19 particles are equally well detected. For manufacturing, the level of the viral concentration in the control is secured by gravimetrically recorded dilutions from calibrated viral stock solutions stored at –70°C.
The external run control tubes are barcoded and comparable in size to donor blood collection tubes. After thawing the tubes are ready for use and can be placed at random positions in sample racks of the laboratory instruments.

Traceability of HAV-RNA and Parvo B19-DNA concentration in IU/ml and copies/ml

The P0266 ViraQ Parvo B19-DNA/HAV-RNA Check is prepared from high titre parvo B-19-DNA plasma stock solution and the HAV-RNA HM175/18F tissue cultured HAV strain obtained from Center for Disease Control (Atlanta, USA), both further diluted in plasma negative for other blood borne viruses. The parvo B19-DNA preparation was quantified in IU/ml against the First International Standard in the WHO collaborative study using real-time parvo B19 DNA PCR test (Roche, Mannheim, Germany) and other methods. The HAV-RNA

standard was quantified in IU/ml as published in the WHO collaborative study for establishment of the first international standard for HAV-RNA.
The HAV-RNA quantification in copies/ml is obtained from testing standard dilution series in the Grifols Duplex assay and calculating the 63 % hit rate using probit analysis. Ideally the 63 % hit rate should equal 2 copies/ml which is assumed to be true. The traceability from the viral standard is secured by the gravimetrical dilution records.

Table 2 concentrations in IU/ml and copies/ml

Marker	IU/ml	Copies/ml (95 % C.I.)
Parvo B19-DNA	10.000	Unknown
HAV-RNA	10	61 (45-61)

The availability of both standards is sufficient for long term manufacturing.

Kit Contents

10 Tubes, each containing 1.5 ml P0266 ViraQ Parvo B19-DNA/HAV Check in polypropylene tubes with screw caps and comparable in size to Vacutainer tubes used for donor sample collection. The run control contains no preservatives.

Storage Instructions

The run controls can be stored at or below -30°C during two years to ensure minimal degradation of Parvo B19-DNA and HAV-RNA. Once thawed the run control samples should be used immediately. Do not refreeze the controls after thawing. Any control sample that appears cloudy or contains precipitates after thawing and mixing should be discarded.

Warning and precautions

P0266 ViraQ Parvo B19/HAV Check contains HAV and Parvo-B19 particles, which are neutralised by antibodies. However the plasma may still be potentially bio-hazardous (see MSDS). The matrix is prepared from human blood plasma that tested negative for blood borne viruses (HAV-RNA,HBV-DNA, HCV-RNA, HIV-RNA, Parvo B19-DNA, HBsAg, 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. Observe the universal precautions for prevention of transmission of infectious agents when handling these materials[8,9].

- 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 Parvo/HAV 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.

Test Procedure

- Thaw the run control quickly in a water bath at 37°C, by gently mixing until frozen contents are **just** thawed.
- **Remove** the run control tube from the water bath **immediately**.

- 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 run control. When not used immediately place in refrigerator. The duration is limited to 8 hours storage until use.
- The controls should be handled and tested in a manner identical to that of clinical specimens in the assay procedure being evaluated.
- The external run control tubes are barcoded for automated data-processing.
- The run controls can be placed at random positions in sample racks of the equipment.
- After testing discard the remaining run control.

Note: Do not refreeze!

Expected assay response

The P0266 ViraQ Parvo B19/HAV Check is expected to react >99.94 % positive for HAV-RNA, including low positive S/CO ratios. The expected proportion low positive S/CO and delta between median and average S/CO will be made available after the PEO phase. In rare cases a nonreactive result can be expected. The P0266 ViraQ Parvo B19/HAV is expected to yield 10.000 IU/ml Parvo B19-DNA (C.I.'s given at performance characteristics).

Note: Final expected assay response values will be made available for the CE marked version of this product.

Interpretation of results

P0266 ViraQ Parvo B19/HAV Check Control should react positive in more than 99.94% of HAV-RNA NAT blood screening test runs and yield a quantitative result for Parvo B19-DNA.

HAV-RNA

In the Duplex assay a S/CO ratio for HAV-RNA above 2.6 is expected on the run control samples. Lower S/CO ratio's are rare, and can be considered as an deviating result. The Westgard[7] rules provide guidance for the interpretation on frequency of lower S/CO results. Trends can be identified by following the difference between average and median S/CO. An increasing difference is indicative for an lower performance.

Parvo B19-DNA

For Parvo B19-DNA the quantitation should be 10.000 IU/ml (99% confidence interval is 4700-21000 IU/ml). Use the Westgard rules and Levey-Jennings plots on log(IU/ml) results to identify aberrant trends.

There is a small probability of non-reactive results, but these can also be related to incidental or systematic errors in the test system. Thus a non-reactive result should be investigated when it occurs. Repeatedly low or nonreactive results on the run control may indicate systematic or random errors in execution of the assay on the automated NAT screening systems and should trigger a root cause analysis.

Note: Final interpretation rules will be made available for the CE marked version of this product.

Performance characteristics

Performance characteristics are under evaluation within this PEO phase. Some HAV related preliminary results are already available, are given below and are for reference purposes only. Upon CE marking of the product the data obtained during the PEO phase will be added to this section for completion.

Analytical sensitivity Grifols Procleix Duplex for HAV-RNA

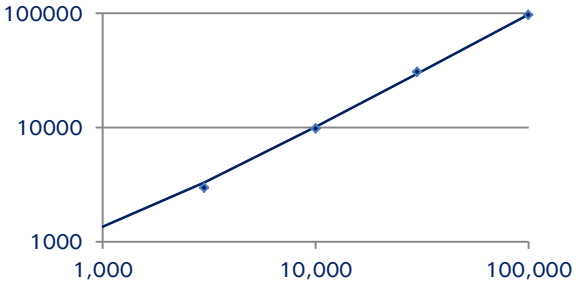
The analytical sensitivity of the Ultrio assay versions is evaluated in different validation studies. Table 3 compares the 50% and 95% LODs in these studies showing consistent analytical sensitivity on the different HAV-RNA standards.

Table 3. Analytical sensitivity of Procleix Duplex version on BQC HAV-RNA standard

HAV-RNA standard	Assay version	n	50 % LOD (CI) IU/mL	95 % LOD (CI) IU/mL	Origin
BQC secondary HAV genotype 1a #	Grifols Procleix Duplex	7	0.189 (0.084-0.388)	1.401 (0.657-4.128)	Hologic
WHO International standard		24, 27	0.248 (0.176-0.322)	1.227 (0.900-1.956)	IHIT, FRC

Correct quantification at of Duplex assay at 10.000 IU/ml

Figure 1 depicts the linearity of the duplex assay on the BQC parvo B19-DNA genotype 1 dilution series (mean value, n = 100, 2 sites, 3 reagent lots on four different dilutions).



Run controls containing 10.000 IU/ml were tested from 2013 to 2015, n = 439. Figure 2 present a levey-jennings chart. Geometrical average is 9960, 95 % confidence interval: 5925-16745 and 99 % confidence interval, 4716-21037 IU/ml

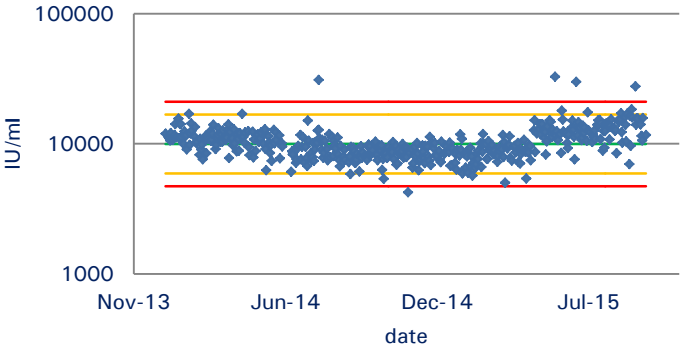


Figure 2 Levey-Jennings chart Parvo B19-DNA quantification

Limitations

- P0266 ViraQ Parvo B19/HAV Check cannot be used to evaluate the analytical or diagnostic sensitivity or accuracy of NAT blood screening assays.
- P0266 ViraQ Parvo B19/HAV Check must not be substituted for the mandatory controls or calibrators provided with IVD test kits for calculating the cut off and/or criteria for releasing test results.
- The response values on the run controls should not be used for a decision to accept or reject the test run.
- The expected distributions of assay response values on P0266 ViraQ Parvo B19/HAV Check that presented in this package insert were based on evaluation studies involving a limited number of assays and reagent batches. Therefore it cannot be guaranteed that slightly different results will be found on other assay versions or reagent batches.

References

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3. A World Health Organization International Standard for hepatitis A virus RNA nucleic acid amplification technology assays. Saldanha J, Heath A, Lelie N, Pisani G, Yu MY; Collaborative Study Group. Vox Sang. 2005 Jul;89(1):52-8
4. <10.000 IU/ml B19V DNA in starting pools Human anti-D immunoglobulin (EP monograph 557)
5. <10.000 IU/ml B19V DNA, and HCV-RNA and HAV-RNA negative (<100 IU/ml) in Human PRT pooled plasma (EP monograph 1646)
6. EP chapter 2.6 21 Guidelines for NAT validation (HCV, B19V, HAV)
7. FDA guideline July 2009 for B19V for in process testing
8. 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.
9. 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.

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