

Performance Characteristics of a MultiCode®-RTx PCR Assay for the Quantitative Detection of



Parvovirus B19 DNA in Serum and Plasma Samples

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Abstract

Parvovirus B19 causes a variety of diseases in humans, including erythema infectiosum, chronic anemia and fetal hydrops. Detection and quantitative monitoring of Parvovirus B19 viremia is a potentially valuable tool both for diagnosis of acute infection, and for monitoring viral activity in chronically infected, immunosuppressed, individuals. We report here on the performance characteristics of a quantitative PCR assay for Parvovirus (qParvo-PCR) that utilizes MultiCode®-RTx technology (EraGen Biosciences, Madison, WI) for detecting PCR products in real-time. One of the advantages of MultiCode®-RTx is that, since tagged-primers rather than probes are used for amplicon detection, amplification efficiency is the sole variable influencing the kinetics of signal generation resulting in improved precision of target quantitation. The qParvo-PCR assay described here enables multiplexed detection of Parvovirus B19 and an extractable internal control target. Quantitation is accomplished via interpolation of results into externally-generated, lot specific, calibration curves. We have established the analytical performance metrics of the qParvo-PCR assay, including the specimen-type specific lower limits of quantitation (LLOQ) and detection (LOD), and delineated the sample storage requirements, reproducibility, specificity and internal control performance of this novel assay. The results of these experiments indicate that this assay is capable of highly reproducible quantitation of Parvovirus DNA in serum and plasma samples over a range of at least 4 logs.

Methods

In brief, the qParvo-PCR assay was performed as follows. Nucleic-acid was recovered from clinical samples (200 µL initial volume) using the Total Nucleic Acid kit on the MagNA Pure LC Instrument (Roche). Co-extraction and recovery of the internal control target (ICT) was performed via addition of ICT to sample (designed to result in a final [ICT] in PCR of 250 cp/rxn). Amplification and detection was performed on an ABI 7900HT instrument with 2-channel detection (FAM for Parvovirus target and JOE for ICT). Positive reactions were considered to be those that yielded Ct values of ≤38 cycles with peak Tm values of 79.3°C – 82.3°C for Parvovirus target and 76.8°C – 79.8°C for ICT. Quantitation of target was accomplished via interpolation of Ct values into externally-generated standard curves constructed using quantitated DNA as the calibrating material.

Sample-type specific LOD and LLOQ values for the qParvo-PCR assay were determined by testing and analyzing multiple replicates (n=24) of a limiting dilution series of Parvovirus positive sample material diluted in pooled negative serum or plasma. Linearity of the assay was assessed for serum and plasma samples by testing serially diluted Parvovirus positive samples.

To establish qParvo-PCR specific conditions for sample storage, serum and plasma samples (n=3) were spiked with Parvovirus positive sample at a final concentration approximately 1 log₁₀ above the LLOQ (5000 cp/mL) and tested in triplicate after storage for 0, 1, 3, 5, 7 and 14 days either refrigerated (2°C to 8°C) or frozen (-15°C to -30°C).

Exclusivity was established by testing high-titers of related viruses, as well as viruses and bacteria potentially present in samples intended for use in the qParvo-PCR assay.

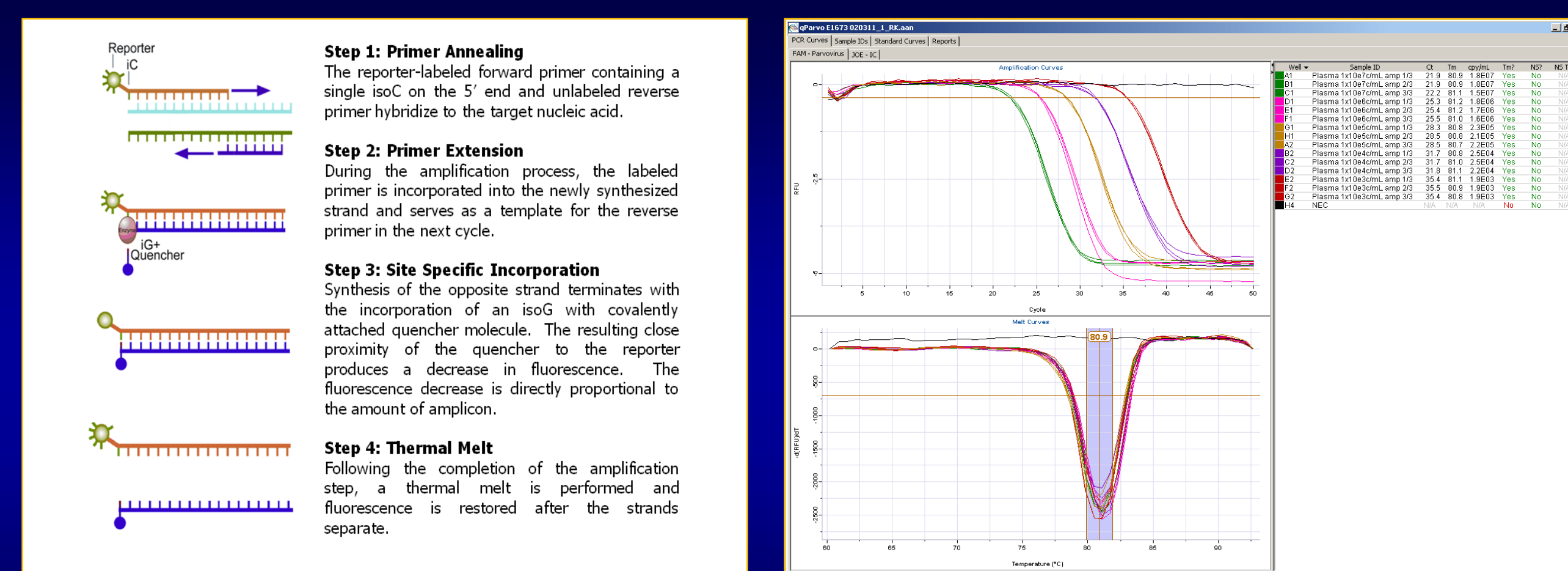


Figure 1. Schematic illustration of MultiCode®-RTx system and instrument readout from qParvo-PCR target dilution series.

Results

Sample Type	Nominal conc. (cp/mL)	No. tested	No. positive	% positive
Serum	1000	24	24	100
	500	24	24	100
	250	24	24	100
	100	24	15	62.5
	50	24	9	37.5
Plasma	1000	24	24	100
	500	24	24	100
	250	24	21	87.5
	100	24	21	87.5
	50	24	21	87.5

Table 1. Determination of sample-type specific LOD for the qParvo-PCR assay. Probit analysis on data shown indicated LOD values for serum of 402 cp/mL and plasma of 139 cp/mL based on a 95% probability of a positive result.

Sample Type	Nominal conc. (log ₁₀ cp/mL)	Mean conc. (log ₁₀ cp/mL)	SD ¹	% CV ²
Serum (n=24)	1000 (3.0)	1316 (3.12)	0.15	38.6
	500 (2.7)	694 (2.84)	0.21	41.0
Plasma (n=24)	1000 (3.0)	1411 (3.15)	0.13	29.3
	500 (2.7)	692 (2.84)	0.21	42.0

Table 2. Establishment of LLOQ for the qParvo-PCR assay. Analysis of quantitative values obtained for a subset of samples in Table 1 indicated reproducible results could be obtained for plasma and serum samples containing a minimum of 500 cp Parvovirus DNA/mL.

¹Using lognormal distribution
²Using normal distribution

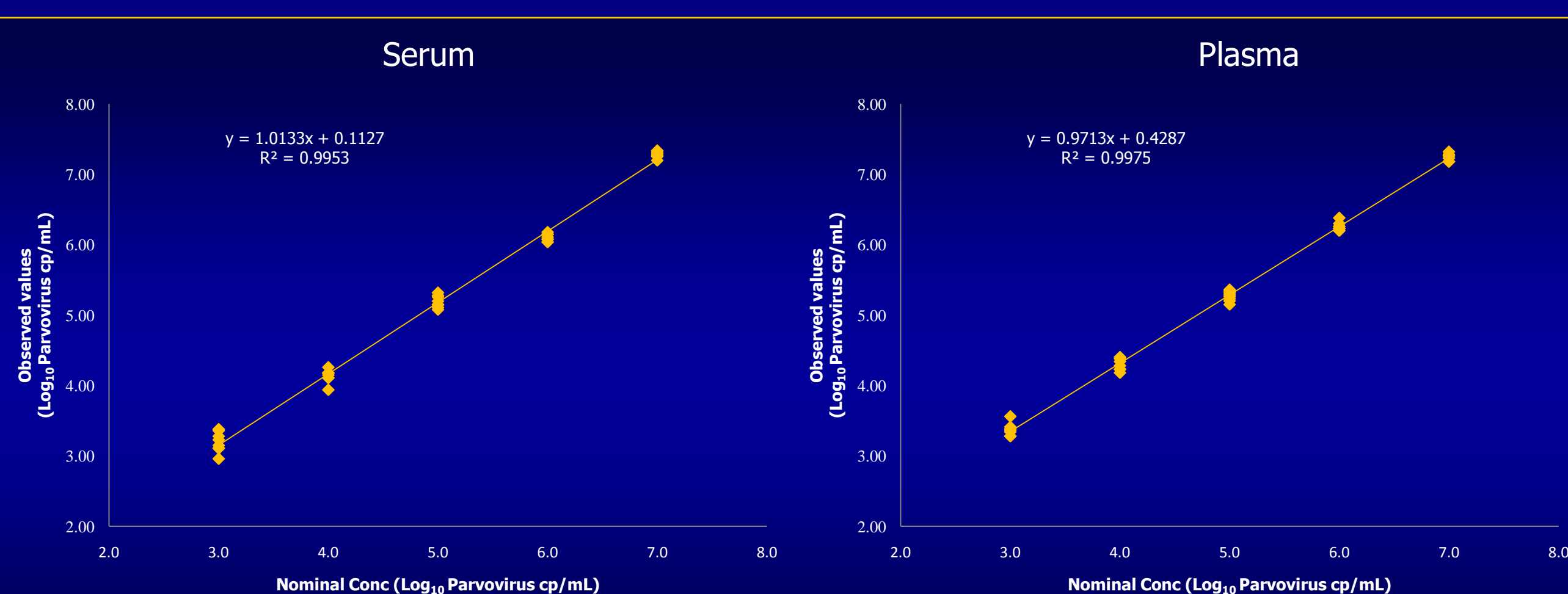


Figure 2. Demonstration of linearity and repeatability of the qParvo-PCR assay for serum and plasma samples. Serum/plasma sample panel consisted of diluted positive clinical samples.

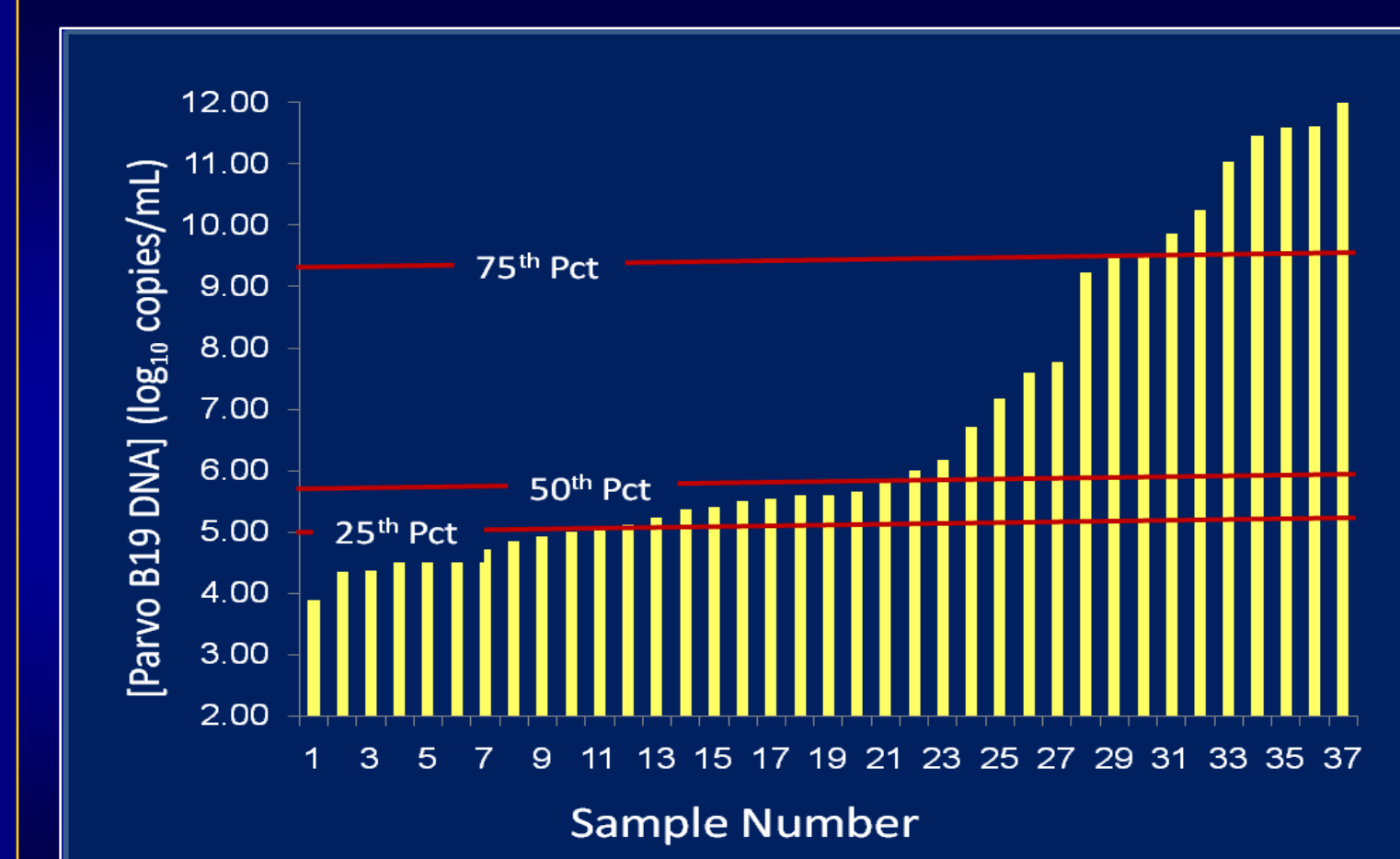


Figure 3. Distribution of Parvovirus B19 viral loads in 37 consecutive serum samples determined to be positive by qualitative PCR analysis. Range of derived values was 7.98 x 10³ cp/mL to >9.0 x 10¹¹ cp/mL, the median viral load was 3.98 x 10⁵ cp/mL, and the mean viral load was 7.08 x 10⁶ cp/mL.

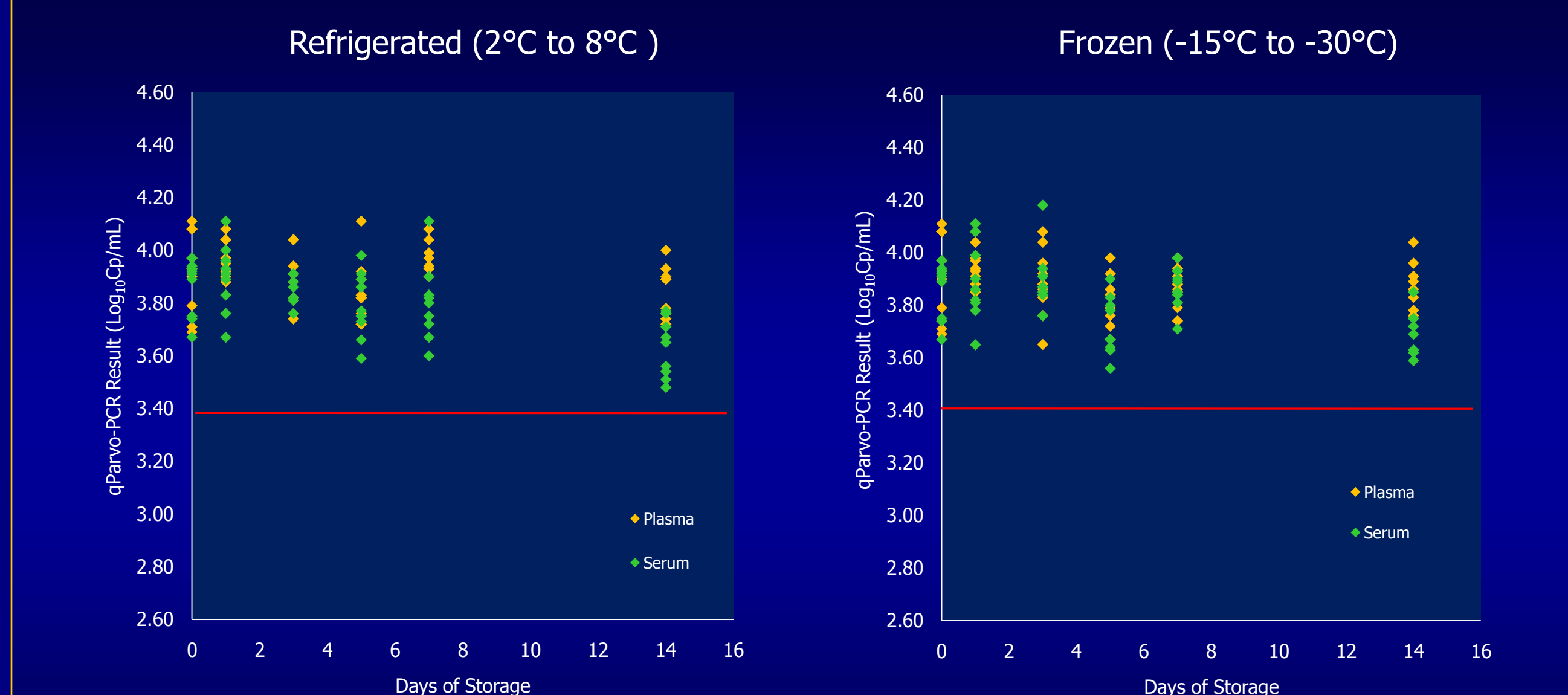


Figure 4. Stability of Parvovirus DNA in serum and plasma samples at refrigerated and frozen temperatures. Samples (n=3 for each sample type) were analyzed in triplicate at each time point. Red bar indicates stability guardband (0.5 log₁₀ cp/mL below mean value at time zero).

Table 3. Exclusivity panel. Table 3 shows organisms tested for cross-reactivity in the qParvo-PCR assay. Testing was performed either on high-titer cultured organisms* or on viral nucleic-acid. All analytes tested generated negative results.

HSV 1	HSV 2	CMV	EBV
VZV	HIV-1	HBV	HCV
<i>E. coli</i> *	<i>K. pneumoniae</i> *	<i>S. aureus</i> *	<i>C. albicans</i> *

Conclusions

- ❖ The results contained here demonstrate the performance characteristics of a qParvo-PCR assay using MultiCode®-RTx chemistry (EraGen Biosciences). The assay, as configured at ViroMed Laboratories, has an LOD of approximately 400 cp/mL for serum and 140 cp/mL for plasma, with an LLOQ of approximately 500 cp/mL for both sample types based on an acceptable total precision of SD ≤0.2 log₁₀ Parvovirus DNA cp/mL.
- ❖ The assay was demonstrably linear over at least 4 logs of target input DNA concentration resulting in an upper limit of quantitation of at least 10 million cp/mL.
- ❖ Distribution of Parvovirus B19 viral loads were analyzed in 37 consecutive serum samples determined to be positive by qualitative PCR analysis. The range of derived values was 7.98 x 10³ cp/mL to >9.0 x 10¹¹ cp/mL, the median viral load was 3.98 x 10⁵ cp/mL, and the mean viral load was 7.08 x 10⁶ cp/mL.
- ❖ Values obtained in the qParvo-PCR assay were not adversely affected by storage of samples for up to 14 days under either refrigerated or frozen conditions.