Comparative Clinical Verification of PCRun® Parvovirus Molecular Detection Kit

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Aim: To determine the sensitivity and specificity of Biogal's PCRun® Parvovirus Molecular Detection Kit as compared to two different RealTime TaqMan PCR protocols utilizing archived samples.

Study design: Retrospective study utilizing archived specimen.

Sample: Fourteen plasma samples collected during a previous vaccination challenge study (2010/unpublished study) performed at the University of Wisconsin and maintained at -80^{0}C prior to extraction. Samples were blinded to the study and labelled 1-14. Details on the vaccination challenge study are noted below.

Outline of the original challenge study: this study was conducted under Institutional Animal Care and Use Committee approval, and was designed to determine onset of protection against CPV-2c challenge following vaccination of naïve dogs with a commercially available CPV-2 vaccine, 48, 72, and 96 hours before challenge. Dogs were randomized and group housed. The challenge organism was isolated from a dog showing signs of Parvovirosis in a shelter in Arkansas. The isolate was typed as 2c at the University of Georgia. Samples (buffy coat, plasma and fecal swabs) were collected at daily intervals which were maintained at -80^{0}C after processing. PCR testing of samples was originally completed at the Wisconsin Veterinary Diagnostic Laboratory (WVDL). Clinical observations were made twice daily. All three vaccinated dogs showed no signs of parvoviral disease, unvaccinated controls showed signs including diarrhea, lethargy, anorexia, dehydration and to a lesser extent bloody diarrhea and vomiting.

PCRun Molecular Analysis of Parvo infection (Feb. 2018):

DNA Extraction Method: One hundred microliters of frozen plasma from the study above were processed employing Zymo Quick-DNA Miniprep Kit (Zymo Research Corp., USA) according to the kit instructions.

Tests performed: (1) PCRun® Canine Parvovirus DNA Detection Kit reactions were performed according to the standard PCRun® protocol employed for extracted DNA. Incubation was performed on a PCRun® Reader at 60^{0}C for 25 min.

(2) Parvo TaqMan Real Time PCR directed at a 113 bp section of the VP2 gene employing LightCycler® 480 Probe Master (Roche Molecular Systems, Inc) was performed on a Mx3000P qPCR System (Agilent). Cq values of 25 and less were defined as positive according to previous studies.

(3) Real Time PCR was performed at WVDL at the time of the study (2010). The results are recorded on Table1.

Table 1: Results of PCRun®, RealTime PCR previously performed at WVDL and RealTime TaqMan performed during the present study

	Plasma				
Sample #	ID	PCRun (TTP)	Real Time (Cq)	WVDL (Cq)	Clinical
1	KZY D0*	0	0	-	Vaccinated, never sick
2	LFZ D0	0	0	-	Unvaccinated control
3	LOZ D0	0	0	-	Unvaccinated control
4	LFZ D4	10	22.28	13.8	Diarrhea
5	KZY D4*	0	0	36.2	Vaccinated, never sick
6	LOZ D4	9	15.41	13.8	Diarrhea
7	KYY D0	0	0	-	Unvaccinated control
8	KYY D4	11	18.92	14.7	Diarrhea
9	PBZ D4	0	0	30.4	Vaccinated, never sick
10	KWZ D4	12	17.47	14.6	Diarrhea
11	LXZ D4	13	22.28	17.5	Diarrhea
12	PBZ D0	0	0	-	Vaccinated, never sick
13	KWZ D0	0	0	-	Unvaccinated control
14	LQZ D4	0	0	30.4	Vaccinated, never sick

*D0 = Challenge Day D4 = Post Challenge day 4

Dogs KZY, PBZ and LQZ were vaccinated with MLV CPV vaccine at 48, 72 and 96 hours (respectively) before challenge.

Dogs LFZ, LOZ. LXZ, KYY and KWZ were unvaccinated and given the same challenge.

Discussion: PCRun® reaction are calibrated to run for 25 min and the RealTime TaqMan or 25 cycles. Any results that fall into this category are considered positive for infectious canine parvovirus and not remnants of vaccination.

1. When using the TaqMan RealTime assay as the Gold Standard for comparison, the sensitivity, specificity and accuracy of the PCRun® reaction were 100%.
2. Comparison of the PCRun results with the clinical status of the animals give 100% correlation.
3. A composite analysis of the clinical status revealed that the same analysis should be applied to the Cq values derived from the WDVL Real Time. With this consideration the PCRun® results correlated positively (100%) with the positive and negative values of the reference lab.