Comparative Clinical Verification of PCRun® Canine Distemper RNA Molecular Detection Kit

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Aim:

The aim of the study was to determine the sensitivity and specificity of Biogal's PCRun® Canine Distemper RNA Molecular Detection Kit as compared to the commercial Reverse Transcription (RT) Distemper Kit designed by Primerdesign ™ Ltd (Cambridge UK) and the in house RT TaqMan PCR employed by Wisconsin Veterinary Diagnostic Laboratory (University of Wisconsin).

Sample type: Thirty-five (35) nose swabs collected from canines suspected of being exposed to Distemper Virus. The samples were accumulated at the Wisconsin Veterinary Diagnostic Laboratory (WVDL), added to carrier medium and maintained at -80^{0}C prior to extraction. Samples were labelled 1-35.

Extraction Method: RNA was extracted from 140 µl carrier medium using QIAmp Viral RNA Mini Kit (Qiagen, Germany) according to the kit instructions.

Tests performed: (1) PCRun® Canine Distemper RNA Detection Kit according to standard protocol employed for extracted RNA. Incubation was performed on a PCRun® Reader for 60 min at 60^{0}C. Results generated by the reader were recorded as minutes "Time to Peak" (TTP).

(2) RT TaqMan PCR amplification was performed on a Mx3000P (Agilent) qPCR System using RT PCR reagents designed by Primerdesign ™ Ltd. (Primer Design Distemper Kit and Oasig RT qPCR Buffers). Cycle threshold values (Ct) were reported as cycle number and copy number values were determined from the Primerdesign™ Positive Control titration curve.

(3) Ct values for the Real Time PCR performed at WVDL were revealed at the end of the study.

Results: Table I contains sensitivity and specificity analysis results. (Ct, TTP and copy number values for each individual sample are displayed in Table II.)

Table I: Comparison of PCRun® results to Primerdesign and WVDL (95%=CI)

	Gold Standard Compared to PCRun®	
	Primer Design (%)	**WVDL (%)**
Sensitivity	100	100
Specificity	100	100
Accuracy	100	100
Pos. Predictive Value	100	100
Neg. Predictive Value	100	100

	Biogal	Primerdesign	RT-PCR (Ct)
Sample #	PCRun® (TTP- min)	RT TaqMan-PCR (Ct)	28.8
1	36	31.1	30.7
2	36	30.8	18.4
3	8	16.6	0
4	0	0	0
5	0	0	28.1
6	38	30.7	28
7	31	28.2	28.7
8	22	30.4	0
9	0	0	0
10	0	0	23.1
11	15	23.5	27.4
12	12	28.5	30.1
13	18	33.5	0
14	0	0	23.8
15	15	23.3	0
16	0	0	28.2
17	44	28.6	20.7
18	12	20.3	26.3
19	14	27.7	19.3
20	12	21.1	23.5
21	18	23.8	23.5
22	13	23.4	0
23	0	0	24.8
24	14	23.4	20.8
25	15	21.3	19.7
26	12	20.73	21.2
27*	0	0	0
28	0	0	29.5
29*	0	0	26.4
30	30	27.7	0
31	0	0	20.2
32	16	22.1	0
33	0	0	0
34	0	0	0
35	0	0	

Discussion and Conclusions: *Samples # 27 and 29 were negative by Primerdesign and PCRun® testing. The amplifications were repeated in order to determine if a technical error had occurred on the first test, but the samples remained negative. They were not included in the final analysis seen in Table I. It must be noted that the RNA samples used in the WVDL testing were not the same as the one used in this study. It can be assumed that the sample was compromised prior to RNA extraction and therefore the tests were negative. The negative results cannot be accounted to low efficiency of the two tests.*

PCR® Canine Distemper RNA Molecular Detection Kits demonstrates very high sensitivity and specificity in the detection of Distemper Virus in nasal swabs collected from canines demonstrating symptoms of the disease.