Canine Distemper RNA Molecular Detection Kit

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Aim: 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).

Background: Canine distemper is a highly contagious lethal viral disease occurring in both domestic and wild canids worldwide. The virus is transmitted by aerosols and direct contact, resulting in fever, serous nasal discharge and cough, as well as respiratory, neurological and gastrointestinal signs complicated by secondary bacterial infections. Molecular tests such as the Polymerase Chain Reaction (PCR) are the most accurate method to diagnose the disease[1]. Primerdesign Real Time PCR and Biogals's isothermal PCRun Distemper RNA Detection Kit are both PCR platforms designed to detect the nucleocapsid (N) gene in clinically derived samples from patients demonstrating acute signs of the Distemper virus.

Method: RNA was extracted from thirty-five (35) nasal swabs collected from canines suspected of being exposed to Distemper Virus and stored at the Wisconsin Veterinary Diagnostic Laboratory (WVDL), The swabs were maintained in carrier medium and stored at -80° C prior to extraction. The extraction process was carried out on 140 µl carrier medium using QIAmp Viral RNA Mini Kit (Qiagen, Germany) according to the kit instructions.

Two molecular amplifications methods were employed to analyze the RNA extracts: (i) PCRun® Canine Distemper RNA Detection Kit according to standard protocol. Incubation was performed on a PCRun® Reader for 60 min at 60° C. Results generated by the reader were recorded in minutes as "Time to Peak" (TTP) values; (ii) Real Time 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.

Results: The results generated from the 35 samples analyzed using PCRun were compared to those generated from the Primerdesign RealTime PCR.

The following observations were noted:

1. In total 21 out of the 35 samples tested positive for Distemper virus by both methods.

2. No false positives or false negatives were observed with the PCRun Distemper kit.

3. TTP values generated by the PCRun tests ranged between 8 – 44 minutes with an average value of 20.5.

4. The Real Time Cq generated with the Primerdesign reactions ranged between 16.6 - 33.5 cycles with an average of 25.6.

Calculations for sensitivity specificity and accuracy are summarized below in tables 1a and 1b.

Table 1a Number of positive and negative results for each test

		TaqMan Reference		
PCRun DNA test kit		Positive	Negative	Total
	Positive	21	0	21
	Negative	0	14	14
	Total	21	14	35

Table 1b Calculated Results for PCRun (%)

TEST	Sensitivity	Specificity	Accuracy	Disease Prevalence	Positive Predictive Value	Negative Predictive Value
Canine Distemper	100.0	100.0	100.0	60.0	100.0	100.0

Discussion and Conclusions:
The 35 nasal swabs samples collected at the Wisconsin Veterinary Diagnostic Laboratory from canines suspected to have been exposed to the Distemper virus were tested employing Primerdesign Real Time PCR and Biogal's point-of-care PCRun Canine Distemper RNA Detection Kit.

There was complete agreement between the Real Time TaqMan PCR and the PCRun amplifications. When applying the Real Time PCR as the Criterion Standard, PCRun had a sensitivity of 100% and a specificity of 100%. Screening of the RNA extracts with the PCRun detected a prevalence of 60% in the population examined, giving a positive predictive value of 100% and a negative predictive value of 100% for the PCRun test.

The PCR® Canine Distemper RNA Molecular Detection Kits demonstrated excellent sensitivity and specificity in the detection of Distemper Virus in nasal swabs collected from canines demonstrating signs of the disease.

Reference:
Beniam Degene and Moges Zebene. (2019). Canine Distemper, A Review. Int. J. Adv. Res. Biol. Sci. 6(7): 12-19.

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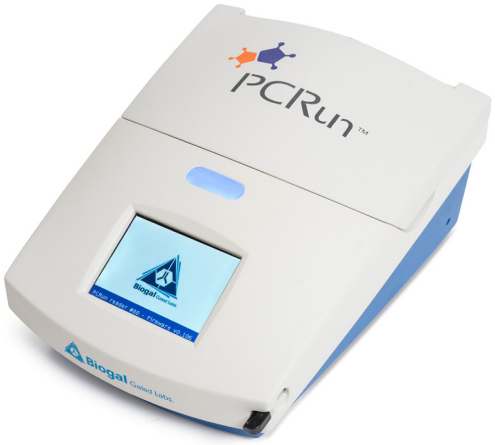

An isothermal amplification detection kit. Delivers a PCR test result in 75 min.