Analysis of PCRun® Canine *Babesia canis* Molecular Detection Kit

Compared to TaqMan RealTime PCR

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Aim: To determine the sensitivity, specificity and accuracy of Biogal's Canine *Babesia canis* Molecular Detection Kit.

Background: Canine babesiosis refers to a tick-borne infection caused by a microscopic parasite that infect red blood cells. PCRun® Canine *Babesia canis* Molecular Detection Kit is based on isothermal nucleic acid amplification technologies that targets part of the 18s rDNA gene of *Babesia canis* and *vogeli*. It designed for the qualitative detection of *B. canis canis* and *B. canis vogeli* in extracted DNA and can be employed for the detecting the acute phase of babesiosis resulting from these organisms.

Method: Between the years, 2015 and 2016, three hundred canine whole blood samples were collected in EDTA from patients which visited the Pendragon Animal Health Group Inc. clinic, Manila Philippines. The samples were frozen and shipped to Biogal Galed Lab Acs, Israel for further testing.
One hundred µl of each defrosted blood sample were used to manually extract 200 µl DNA using DNeasy Blood & Tissue Kit (Qiagen, USA). Purified DNA samples were maintained at -20^{0}C prior to testing. The extracted DNA was employed for molecular analysis using an in-house species specific TaqMan PCR based on the protocols designed by Michelet et al [1] and Biogal's PCRun® Canine *Babesia canis* Molecular Detection Kit. Two separate TaqMan reactions were performed to differentiate between *B. canis canis* and *B. canis vogeli*. The PCRun® reagents contain primers which identify both species therefore it was necessary to perform only one test per sample.
Amplification of the TaqMan reactions was carried out on a LightCycler® 96 System (Hoffmann-La Roche Ltd). Positive and negative results were determined from the Cq values of the TaqMan and compared to positive and negative controls (molecular grade water and naïve canine DNA).
For the PCRun® reactions, amplification was carried out at a constant temperature of 60^{0}C for 60 min. in a luminescence reader/heater (PCRun® Reader) developed and marketed by Biogal. The PCRun® reader produces a real time graph as well as a tab-oriented positive/negative palette. Results from the PCRun® reactions were defined in time units referred to as Time to Peak (TTP).

Results:

The results generated from the 300 samples tested with PCRun® Canine *Babesia canis* Molecular Detection Kit were compared with those from the TaqMan RealTime PCR. The following observation were observed:

1. RealTime TaqMan tests revealed that all of the positive samples could be classified as *Babesia canis vogeli*. *Babesia canis canis* was not detected in any of the bloods received from the Philippines in this trial.
2. In total, 34 out of the 300 samples were positive for *B. vogeli* as registered by the TaqMan PCR; of these positive samples, PCRun® Canine *Babesia canis* Molecular

Detection Kit returned a positive reaction with 31 samples. Three false negative calls occurred with the isothermal kit.

3. The time range (TTP) to observe a positive result for PCRun® was 20-50 min. with an average TTP of 29 minutes
4. Time range for Real Time (Cq) was 20.5-40 min. with an average Cq of 33 min.
5. No false positives were observed with PCRun® Canine *Babesia canis* Molecular Detection Kit when compared to the TaqMan PCR protocol.

Calculations for sensitivity specificity and accuracy are shown below.

Table 1a and **1b**: Results of sensitivity, specificity and accuracy as determined using the following formula.

Table 2: Calculated Results: Results

Table 1a.

		TaqMan Reference		
		Positive	**Negative**	**Total**
Babesia DNA test kit	**Positive**	a	b	a+b
	Negative	c	d	c+d
	Total	a+c	b+d	a+b+c+d
		% Sensitivity = 100 x a/(a+c)	% Specificity = 100 x d/(b+d)	% Accuracy= 100x(a+d)/(a+b+c+d)

Table 1b.

		TaqMan Reference		
		Positive	**Negative**	**Total**
Babesia	**Positive**	31	0	31
	Negative	3	266	269
	Total	34	266	300
	CI=95%	% Sensitivity = 100 x 31/(31+3)	% Specificity = 100 x 266/(0+266)	% Accuracy= 100x (31+266)/(31+0+3+266)

Table 2: Calculated Results (%)

Test	Sensitivity	Specificity	Accuracy	Disease Prevalence	Pos. Predictive Value	Neg. Predictive Value
Babesia canis (vogeli)	**91.9**	**100.0**	**99**	**12.21**	**100.0**	**98.9**

[1]. High-throughput screening of tick-borne pathogens in Europe. Frontiers in Cellular and Infection Microbiology (July 2014, Vol 4, Article 103). Michelet et al.