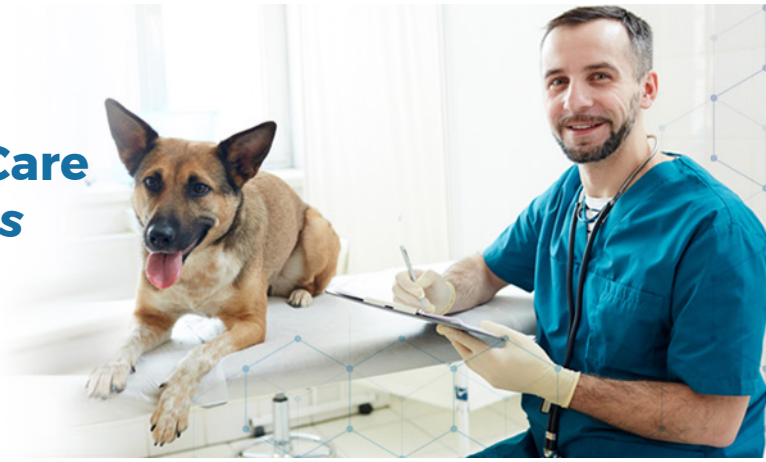
PCRun™ a Novel Point-of-Care PCR for the *Ehrlichia canis*



Tal Yaaran, Kirsty Davies, **Sarah Maurice**, Guy Kiddle.
Biogal Galed Labs ACS, Galed Israel;
Lumora Ltd, Ely UK.

PCRun® by Biogal Galed Labs.

Background: Ehrlichia canis is an obligately intracellular, tick-transmitted (Rhipicephalus sanguineus), gram-negative, bacterium. Following transmission, the rickettsia reside within monocytes and macrophages as microcolonies within intracellular vacuoles (morula). E. canis is the primary etiologic agent of canine monocytic Ehrlichiosis with distribution in the United States, Europe, South America, Asia and the middleast. With adequate treatment, dogs typically recover, but when untreated or inappropriately treated they can develop subclinical persistent infections and become asymptomatic carriers1.
Molecular methods, such as Polymerase Chain Reactions (PCR), deliver rapid, sensitive and highly specific detection of targeted pathogen nucleic acids. Diagnosis can be accomplished in the early stages of disease, well before antibodies are detectable.

Aim: The aim of the study was to determine the sensitivity, specificity and accuracy of Biogals's isothermal Ehrlichia canis PCRun®DNA Detection Kit which targets a section of the 16S rDNA gene. This was accomplished by comparing the performance of the kit with an in-house probe-based TaqMan Real Time PCR which targets the citrate synthase gene (glt4)[2].

Method: The test samples consisted of 33 EDTA whole blood samples provided by Dr Tristan Cogan at the School of Veterinary Sciences (Bristol University, UK) and a further 215 samples collected from dogs in Israel displaying anorexia, elevated temperature and thrombocytopaenia along with a history of recent exposure to ticks. DNA extractions were performed using DNeasy Blood and Tissue Kit (Qiagen).
Primer sequencing and amplifications of the Real Time method is described in Thomson et al3.
Amplification reactions were carried out on a LightCycler® 96 System (Hoffmann-La Roche Ltd). Positive and negative results were determined from the Cq values derived following comparison to positive and negative controls (validated positive extracts and naïve canine DNA). The PCRun® reactions, amplification was carried out according to the manufacturer's instructions at a constant temperature of 60 0C for 60 min. in a luminescence reader/heater (PCRun Reader) developed and marketed by Biogal. Results from the PCRun reactions were defined in time units (min) referred to as Time to Peak (TTP). PCRun results were recorded on a PCRun™ Reader followed by analysis with Ustar DNA Detection Devices.

Results: The Cq-values generated using the gltA TaqMan method were compared with TTP readings on the PCRun Reader, and end-point analysis using Ustar Nucleic Acid Detection Devices. Test comparisons were performed on the positivity of data using MedCalc Software.

1. 14 out of the 33 samples tested from Bristol University were positive for *E. canis* as registered by both the Real Time PCR and the PCRun Ehrlichia canis Molecular Detection Kit.

2. 45 out of the 215 Israeli samples were positive for *E. canis* as registered by both the Real Time PCR and the PCRun Ehrlichia canis Molecular Detection Kit

3. No false positive occurred with the isothermal kit.

4. The time range (TTP) for positive results with the PCRun® reaction was 20-50 min. with an average TTP of 29 minutes

5. Time range for Real Time (Cq) was 20.5-40 min. with an average Cq of 33 min.

Calculations for sensitivity specificity and accuracy of the combined sample groups (N=248) are summarized below in tables 1a and 1b below.

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

	TaqMan Reference	
PCRun	Positive	Negative
	59	0
	0	189

Table 1b Calculated Results for PCRun (%)

TEST	Sensitivity	Specificity	Accuracy	Positive Predictive Value	Negative Predictive Value
Ehrlichia canis	100.0	100.0	100.0	100.0	100.0

Discussion and Conclusions:
Overall, there was excellent agreement between the results derived from the Real Time TaqMan PCR and the PCRun tests. The data presented above show that the PCRun and TaqMan assay have excellent correlation for positivity and negativity. This work demonstrates that both diagnostic tools have similar sensitivities with respect to the field samples studied despite differing target sequences and geographical locations. The prevalence of Ehrlichiosis in suspected dogs was concordant with respect to the amplification and geographical location, demonstrating conservation of the respective targets and primer sequences within the populations of dogs tested. The specificity of each test with respect to primer interactions was also very reliable, as no false positives were called throughout the evaluation of either test.
When applying the Real Time PCR as the Criterion Standard, PCRun had a sensitivity of 100% and a specificity of 100%. Screening of the DNA extracts with the PCRun detected a prevalence of 42% for the British samples and 21% for the Israeli samples with a positive predictive value of 100% and a negative predictive value of 100%.

References:
1. Waner, T.1 * and Harrus, S. (2003). Canine Monocytic Ehlrichiosis – From Pathology to Clinical Manifestations. Israel Journal of Veterinary Medicine. Vol. 68 (1), March 2013
2. Roux V., Rydkina E.,Eremeeva M., Raoult D. (1997) Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae.
Int. J. Syst. Bacteriol. 47:252-261.
3. Thomson K., Yaaran T., Belshaw A, Curson L., Tisi L., Maurice S., Kiddle G. (2018). A new TaqMan method for the reliable diagnisi of Ehrlichia spp. in canine whole blood. Parasites & Vectors 11:350.

PCRun®
by Biogal Galed Labs.

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