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

BACKGROUND

Ehrlichia canis is an obligately intracellular, tick-transmitted (*Rhipicephalus sanguineus)*, gram-negative, bacterium classified in the order *Rickettsiales*. Following transmission, the rickettsia reside as microcolonies within intracellular vacuoles (morula), within monocytes and macrophages of mammalian hosts. *E. canis* is the primary etiologic agent of canine monocytic Ehrlichiosis with distribution in the United States, Europe, South America and Asia [1].

The infective agent is maintained in nature by persistent passage in wild and domestic canids. Ehrlichiosis is considered to be an emerging life-threatening anthropozoonoses. With adequate treatment, dogs typically recover, but when untreated or inappropriately treated they can develop subclinical persistent infections and become asymptomatic carriers.

The indirect fluorescent-antibody assay (IFA) is the diagnostic gold standard diagnostic protocol, but this test can be performed only in specialized laboratories and interpretation of the results is subjective. 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. Biogal ACS Galed Labs have developed a novel isothermal PCR (PCRun™) kit, Canine Ehrlichia Molecular Detection Kit, directed at part of the 16S rRNA gene of *E. canis.*

This kit can be accompanied by a sample prep (Biogal PCRun™ Rapid DNA Extraction Kit) which requires minimal equipment.

AIM

The aim of the study was to compare the efficiency of PCRun™ DNA Detection Kit with an in-house probe-based TaqMan Real Time PCR . For this purpose reactions were carried out using DNA extracted by PCRun™ DNA or Qiagen DNeasy Blood and Tissue Extraction Kits.

METHOD

<u>Samples</u> From 2011- 2015 whole blood samples were collected from dogs diagnosed with typical clinical signs of Ehrlichiosis and stored at -20^{0}C until DNA extraction.

<u>TaqMan PCR</u>

DNA extraction: DNA was extracted from 100 µl whole blood using Qiagen DNaeasy Blood and Tissue Kit and eluted with 200 µl molecular grade water (MGW).

Amplification: *E. canis* DNA was detected by Taq Man Real Time PCR using primers and probes targeting a 146 bp segment of the citrate synthase gene (gltA4FWD 5'-TAGCAACTTTATGGGGGCCA-3'; gltA4REV 5'-TGACCAAAACCCATTAGCCTC-3'; probe gltA4 Prb3 FAM-5'-AGTAACGTAAAGCAGTTTATTCAA-bhq1-3'). The reaction mix consisted of 2x Lightcycler Probes (Roche) master mix with 400 nM of each primer and probe and 5 µl DNA. Amplification was carried out in a Roche Light Cycler® 96 (1 cycle /5 min/95^{0}C; 40 cycles/95^{0}C /15 sec, 55^{0}C/1 min). The negative control contained naive DNA.

PCRun™ Reactions

DNA extraction: A second DNA extraction was performed with 50 µl blood employing PCRun™ DNA extraction kit. DNA was eluted directly into 200 µl of PCRun™ Dilution Buffer.

Amplification: Reactions were performed using the PCRun™ Canine Ehrlichia Molecular Detection Kit. All of the reaction components were present in a freeze dried pellet in 200 µl PCR tubes. Primer sets were designed to target the conserved section of the 16s rDNA gene. The pellet also contained bioluminescent enzyme-substrate reagents for monitoring the amplification reaction.

Amplification reactions were performed using DNA extracted with PCRun™ Prep and Qiagen Dneasy Kit. For Qiagen extracted samples; reaction pellets were dissolved in 15 µl PCRun™ Buffer followed by 5 µl DNA. For PCRun™ prep extractions; pellets were dissolved in 20 µl DNA/ PCRun™ Dilution Buffer. Samples were tested once with positive and negative controls. Incubation occurred at a 60^{0}C (constant)/1 hr. in a PCRun™ Reader. Real time results were observed on the bioilluminator touch screen. Amplified DNA was also analyzed using UStar disposable nucleic acid detection devices.

Analysis of Results

TaqMan - Cq values in comparison with negative controls.

PCRun™ - Time-to-Peak readings on the PCRun™ Reader and verification with Ustar DNA Detection Device

Diagnostic Test Evaluation was performed using MedCalc Software

Comparison of PCR™ DNA Extraction Kit with Qiagen Dneasy Blood and Tissue Extraction Kit.

DNA which was extracted from blood samples (n= 120) using PCR™ DNA Extraction Kit and Qiagen Dneasy Extraction Kit was tested using PCRun™ Molecular Detection Kits. Results were recorded on a PCRun™ Reader followed by analysis with a Ustar DNA Detection Device. The validity of the PCRun™ DNA extraction kit was determined in comparison with Qiagen Dneasy Extraction kit. (CI = 95%)

Sensitivity	96.7%
Specificity	97.8%
Positive predictive value	93.5%
Negative predictive value	98.9%

Comparison of PCRun™ Canine Ehrlichia Molecular Detection Kit and TaqMan PCR (Ustar analysis).

DNA samples extracted from whole blood (n= 175) with Qiagen Dneasy Kit were used for analysis using PCRun™

Molecular Detection Kit and TaqMan Real Time PCR. Cq values within 40 cycles were noted as positive for TaqMan. PCRun™ DNA Detection Kit results were analyzed with a **Ustar DNA Detection Device**. The validity of the PCRun™ Ehrlichia Kit was determined in comparison with Real Time TaqMan PCR. (CI = 95%)

Sensitivity	100%
Specificity	99.3%
Positive predictive value	97.1%
Negative predictive value	100%

Data on File – Biogal Galed Labs

Comparison of PCRun™ Canine Ehrlichia Molecular Detection Kit and TaqMan PCR (PCRun™ Reader analysis).

DNA samples extracted from whole blood with Qiagen Dneasy Kit were used for analysis using PCRun™ Molecular Detection Kit and TaqMan Real Time PCR. Cq values within 40 cycles were noted as positive for TaqMan. PCRun™ DNA Detection Kit results were recorded on a **PCRun™ Reader**. The validity of the PCRun™ Ehrlichia Kit was determined in comparison with Real Time TaqMan PCR. (CI = 95%)

Sensitivity	**97%**
Specificity	**100%**
Positive predictive value	**100%**
Negative predictive value	**99.3%**

DISCUSSION AND CONCLUSIONS

- The prevalence of *E. canis* in blood samples collected from dogs expressing symptoms of Ehrlichiosis ranged between 19-22%. Four of the negative samples were found to be positive for *Anaplasma platys* (results not shown).
- A high level of positive correlation in sensitivity and specificity was found when comparing the two sample prep methods. The PCRun™ DNA Extraction Kit has the advantage that it is very rapid (10 min compare to 25 minutes with Qiagen Prep). In addition the PCRun™ Prep does not require centrifugation.
- Ehrlichia PCRun™ DNA Detection Kit and TaqMan Real Time PCR demonstrated similar detection efficiencies.
- A very slight but not significant difference was seen when using the PCRun Reader or Ustar DNA detection devices.

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

Data on File – Biogal Galed Labs