Background

Forty-five samples of whole blood collected in Israel (30) and Greece (15) from canines suspected of suffering from Canine Leishmaniosis were tested by real-time TaqMan PCR and PCRun® *Leishmania infantum* DNA Detection Kit. Sensitivity and specificity values were calculated using the real-time PCR as the Gold Standard.

Method

Samples

Blood samples were collected into EDTA Vacutainers by means of venipuncture. The samples were aliquoted into working volumes and maintained at -20^{0}C. DNA was extracted from 100 µl of defrosted blood using DNeasy Blood and Tissue Kit (Qiagen). The purified DNA was eluted with 200 µl of EB Buffer (Qiagen) and maintained at -20^{0}C prior to testing.

Real-Time PCR

Real-time TaqMan PCR was performed using the primers and protocol describe in the article: "Detection of Leishmania infantum DNA by real-time PCR in canine oral and conjunctival swabs and comparison with other diagnostic techniques. Veterinary Parasitology 184 (2012, pp 10-17) Solano-Gallegoc L. et al.". Briefly; amplification was carried out in a final volume of 20 µl with 5 µl extracted DNA. Primers and probe were diluted into LightCycler® Probe Master and the reaction carried out in a LightCycler® 96 (Roche) Thermocycler. Positive and non-template controls (molecular grade water and naïve DNA) were added to each trial. Cq values were used to determine whether the samples were positive or negative when compared to the negative controls. In addition, pure DNA extracts for the species *L. infantum*, *major, donavanii and tropicana* acquired from the American Type Culture Collection were added to the test.

PCRun® Leishmania infantum DNA Detection Kit

Standard PCRun® reactions were carried out according to the manufacturer's instructions. Briefly; the reaction pellets were dissolved in 15 µl PCRun® Buffer followed by 5 µl of extracted DNA. Amplification was carried out in a PCRun® Reader for 60 minutes using the test protocol Generic. All amplicons were also analyzed with lateral flow DNA Detection Devices.

Results

Estimates of sensitivity, specificity, accuracy, positive predictive values and negative predicative values were determined using EPR-Val Test Pack Web browser version.

1. The real-time PCR was able to amplify all 4 species of Leishmania, while the PCRun® Detection kit identified the only *Leishmania infantum*.
2. Of the 45 samples tested by real- time, 29 were found to be definitely positive and 16 negative.

The estimated values are noted below:

	%	95%CI
Sensitivity	86.2	68.3-96.1
Specificity	84.6	54.6-98.1
Accuracy	85.7	71.5-94.8
Positive predictive value	92.6	77.6-97.8
Negative predictive value	73.0	51.8-87.5