Inclusivity and Exclusivity Study

PCRun™ Canine Pathogenic *Leptospira* Molecular Detection Kit

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Background

Leptospira **are aerobic, slow growing, fastidious, gram-negative spirochetes which posess spiral corkscrew-like motility.** ***Leptospirosis*** **is caused by the pathogenic** ***Leptospira*** **strains which are not readily distinguishable on the basis of morphology and biochemical characteristics from saprophytic** ***leptospire*** **strains. Traditionally,** ***Leptospira*** **were divided into 2 groups: pathogenic, which were defined as members of** ***L interrogans*** **and saprophytic** ***leptospira,*** **classified with** ***L. biflexa.*** **Based on surface antigen analysis, an approximate 250 different serovars of pathogenic** ***Leptospira*** **have been identified throughout the world. Serovars having antigenic similarities have been formed into 25 distinct serogroups. With the advent of molecular biology, the genus** ***Leptospira*** **has been reorganized within the pathogenic** ***leptospires*****. The serovar names remain and are useful when discussing epidemiology, clinical features, treatment, and prevention of leptospirosis[1,2].**

The PCRun™ Canine Pathogenic ***Leptospira*** **Molecular Detection Kit was developed by Biogal Galed Labs in order to be employed as a Point of Care tool for the detection of** ***Leptospira*** **present in DNA extracted from canine whole blood and/or concentrated urine. The reaction is based on an isothermal polymerase chain process employing primers directed at the hemolysis-associated protein (HapI) encoding gene. Recent studies have determined that this protein is expressed in pathogenic** ***leptospires*** **but not in intermediate or saprophytic isolates[3].**

Aim

The aim of the project was to determine whether PCRun™ Canine Pathogenic ***Leptospira*** **Molecular Detection Kit can (i) efficiently identify pathogenic strains of** ***Leptospires*****; (ii) differentiate pathogens from saprophytes and; (iii) not present cross reactivity with non-specific bacteria which are often present passively in clinical samples. DNA extracted from pure cultures of 34 pathogenic and 3 saprophytic classified serovars donated by Dr. Zbigniew Arent at the OIE** ***Leptospira*** **Reference Laboratory, Agri-Food and Biosciences Institute, Belfast, Northern Ireland were employed in this study. In addition 10 non-specific in-house bacterial isolates were tested.**

Method

Leptospira **DNA extracted using the Qiagen Dneasy blood and tissue kit was analyzed and detected using PCRun™, employing propriety labelled primers designed to target a ≈200 bp section of the HAP1 gene. PCRun™ lyophilized reaction pellets were dissolved in 15 µl PCRun™ Buffer followed by 5 µl**

extracted DNA. Test samples and controls were tested once only. Positive and negative controls (NTC) were added to each test run.

Reactions were carried out at a constant temperature of 60^{0}C (1 hr.) in a PCRun™ Bio illuminator Reader and real time results of amplification were observed on the bio illuminator touch screen.

Positive peaks which appeared within 60 minutes incubation were noted as positive.

Table 1. Inclusivity and Exclusivity of PCRun™ Canine Pathogenic *Leptospira* Molecular Detection Kit. Pathogenic and saprophytic *Leptospira* DNA samples were analysed with PCRun™ Bio illuminator. All previously defined pathogenic serovars were amplified while saprophytic isolates were not. All positive controls were positive and NTCs were negative.

Species	Serovar	Serogroup	PCRun	Phenotype
L. interragans	Icterohaemorrhagiae	Icterohaemorrhagiae	+	
L. interragans	Canicola	Canicola	+	
L. interragans	Pomona	Pomona	+	
L. interragans	Bratislava	Australis	+	
L. interragans	Australis (Ballico)	Australis	+	
L. interragans	Pyrogenes	Pyrogenes	+	
L. interragans	Hebdomadis	Hebdomadis	+	
L. interragans	Wolfii	Sejroe	+	
L. interragans	Hardjo-Prajitno	Sejroe	+	
L. interragans	Autumnalis	Autumnalis	+	
L. borgpetersenii	Sejroe	Sejroe	+	
L. borgpetersenii	Hardjo-Bovis	Sejroe	+	
L. borgpetersenii	Castellonis	Ballum	+	
L. borgpetersenii	Tarassovi	Tarrasovi	+	
L. borgpetersenii	Javanica	Javanica	+	
L. kirschneri	Grippotyphosa	Grippotyphosa	+	
L. kirschneri	Mozdok	Pomona	+	**Pathogenic isolate**
L. kirschneri	Altodouro	Pomona	+	
L. kirschneri	Cynopteri	Cynopteri	+	
L. kirschneri	Valbuzzi	Grippotyphosa	+	
L. noguchii	Louisiana	Louisiana	+	
L. noguchii	Nicaragua	Australis	+	
L. noguchii	Proechimys	Pomona	+	
L. noguchii	Peruviana	Australis	+	
L. noguchii	Orleans	Louisiana	+	
L. weilii	Mengma	Javanica	+	
L. weilii	Sarmin	Sarmin	+	
L. santarosai	Dania	Pomona	+	
L. santarosai	Canalzonae	Grippotyphosa	+	
L. santarosai	Tropica	Pomona	+	
L. inadai	Malaya	Canicola	+	
L. inadai	Shermani	Shermani	+	
L. meyeri	Semaranga	Semaranga	+	
L. meyeri	Sofia	Javanica	+	
L. fainei	Hurstbridge	Fainei	-	**Saprophytic isolate**
L. biflexa	Patoc	Semaranga	-	

L. biflexa	Andamana	Andamana	-	

Table 2. Exclusivity- PCRun™ Canine Pathogenic *Leptospira* Molecular Detection Kit. Non-*leptospire* bacterial DNA samples were examined by PCRun™ analysis with a PCRun™ Bioilluminator. All of the non-specific isolates were negative.

Genus/species	**PCRun**	**Phenotype**
Leptonema	-	**Non specific**
Aeromonas hydrophila	-	
Staphylococcus aureus	-	
Citrobacter freundii	-	
Enterobacter cloaca	**-**	
Klebsiella pneumonia	**-**	
Proteus mirabilis	-	
Salmonella enteritidis	-	
Salmonella livingstone (C)	-	
Escherichia coli	-	

DISCUSSION

DNA samples extracted from previously classified pure *Leptospira* cultures representing 34 different pathogenic and 3 saprophytic serovars were analysed employing PCRun™ Canine Pathogenic *Leptospira* molecular detection technology. The active reagents of the kit were lyophilized in PCR strips, therefore preparation of the reaction only required 2 simple pipetting steps; reconstitution of the the amplification reagent and addition of the DNA. The PCRun™ Reader served as a heat block, that maintained a constant temperature, as well as a bio illuminator. The light intensity was followed as the amplification process progressed; any samples that produced a peak during the 60 minute incubation period were scored positive.

- **NTCs were negative for 60 min and the positive controls were all**

amplified in good time.

- **The HapI DNA target successfully identified all the pathogenic serovars tested without cross reacting with bacteria which can potentially contaminate clinical field samples.**
- **All of the pathogenic serovars were amplified by PCRun™ with a high degree of efficiency, in under 60 minutes, and no false positives were recorded with the non-specific isolates.**

references

1. Antoniadis, A., Alexiou-Daniel, S., Finadin, L. and Bautz, E.F.K. 496. (1995) Comparison of the clinical and serologic diagnosis of 497 haemorrhagic fever with renal syndrome (HFRS) and *leptospirosi**s**. **Eur. J. Epidemiol**. **8, 228–232. 499**

2. Zaki, S.R. and Shieh, W.J. (1995) *Leptospirosis* associated with 503 outbreak of acute febrile illness and pulmonary haemorrhage, 504 Nicaragua, 1996. The Epidemic Working Group at Ministry of 505 Health in Nicaragua. ***Lancet* 24 (347), 535–536.**

3. Christine Branger et al (2005). Polymerase chain reaction assay specific for pathogenic Leptospira based on the gene hap1 encoding the hemolysis-associated protein-1**. *FEMS Microbiology Letters*. Vol 243, Issue 2, pp 437-445.**