Canine Pathogenic Leptospira Molecular Detection Kit

Cat. No.30CLP108
For *in vitro* veterinarian diagnostic use only
User Manual

INTENDED USE

PCRun™ Canine Pathogenic Leptospira Molecular Detection Kit is intended for the detection of pathogenic *Leptospira* in DNA isolated from canine whole blood, bone marrow and concentrated urine. The kit contains all the disposable components required for performing an easy and accurate test.

PRINCIPLE

PCRun™ is a molecular assay based on isothermal amplification of part of the *Hap*I gene. It is intended for the qualitative detection of pathogenic species of Leptospires. This kit is designed to be used with a compatible heat block.

STORAGE AND HANDLING

- Storage 2-25^{0}C (room temperature or refrigerated).
- Avoid exposure to direct sunlight.
- Do not use beyond expiration date stated on the package label.
- Do not freeze!

Precautions:

- The PCRun™ assay is not to be used on the specimen directly. An appropriate nucleic acid extraction method must be conducted prior to using this kit.
- In order to avoid the introduction of contaminating DNA into the reaction, clean laboratory examination gloves must be worn while performing the test.
- Open and remove the PCRun™ reaction tubes from the sealed pouches only immediately prior to their use.
- **Return unused PCRun™ reaction tubes to the original aluminum packet together with the desiccator. Seal with tape.**
- Do not use kit if the pouch or the components are damaged.
- Each component in this kit is suitable for use only with a specific lot number. Components have been quality control approved as a standard batch unit. Do not mix components from different lot numbers.
- Employ accepted sanitary procedures designated for biological and molecular waste when handling and disposing of the reaction tubes.

BACKGROUND

Leptospirosis is a bacterial zoonotic disease caused by pathogenic species of the genus *Leptospira*. Typical pathogenic serovars infecting dogs include *icterohaemorrhagiae, canicola, pomona, bratislava, grippotyphosa* and *autumnalis*[1]. Leptospires, transmitted by direct contact with contaminated sources, can penetrate abraded skin or mucous membranes and disseminate within the host. In canines the motile spirochaetes can be found in the bloodstream in the first 7-10 days after exposure. The septicemic phase is followed by an immune phase which is characterized by the appearance of antibodies and the clearance of *Leptospires* from the bloodstream. The spirochaetes then colonize the renal tubules of chronically infected hosts. Leptospiruria begins approximately in the second week following the onset of symptoms. Maintenance hosts such as dogs typically remain clinically asymptomatic and shed Leptospires into the environment via urine.

DIAGNOSIS

Leptospirosis can be suspected based on clinical signs and results of kidney and liver functions. A conclusive diagnosis is usually made by demonstrating the presence of the bacteria in the samples or by finding increasing levels of antibodies over time. Techniques available include histochemistry, culture, antigen/antibody reactions and molecular based hybridization or amplification. Culture isolation of causative organisms from clinical samples takes several weeks, microscopic analysis is highly subjective and antibodies are detectable in blood only 5-7 days after the onset of symptoms. Serological surveys report more than 20% of examined canine sera contain antibodies specific for pathogenic Leptospira serovars, however it is difficult to correlate serological titres with the prevalence of chronic infections [2].

Molecular amplification is employed for the rapid detection of pathogens involved in acute infections. Protocols can be applied to selectively amplify highly conserved DNA sequences which are present in pathogenic Leptospires, but absent in saprophytic species. Molecular amplification of DNA isolated from clinical samples can rapidly confirm the diagnosis in the early phase of the disease, before antibody titers are at detectable levels and urine samples can be used to identify chronic carriers of the disease [2].

KIT CONTENTS

Components	Contents	Amount
Aluminum pouch **Cat No. 03CLP100**	PCRun™ strip of 8 lyophilized *Leptospira* single reaction tubes	1
Aluminium pouch **Cat No. 03100010**	Disposable nucleic acid detection device.	8
Drop bottle **Cat. No. 33000001**	1X PBS 1.5 ml	1
Drop bottle **Cat. No. 33000010**	10X PBS 1.5 ml	1
Capillary tubes **Cat No. 03200020**	Disposable plastic capillary tubes - 20 µl*	10

***Accurate laboratory pipettes with aerosol barrier tips can be used in place of the plastic capillary tubes.**

EQUIPMENT TO BE SUPPLIED BY USER:

- Biogal PCRun™ Sample Prep
- Heat block which maintains 60^{0}C – compatible with 0.2 PCR tubes. Heat block can be supplied by Biogal
- Timer
- Small scissors
- Fine tipped permanent marker
- Protective laboratory gloves
- For urine samples, microcentrifuge which reaches 10,000 rpm

SAMPLE COLLECTION, STORAGE AND TRANSPORT

The kit is suitable for detecting nucleic acid extracted from 50 µl of whole blood or the pellet derived from concentration of 1 ml of urine using the PCRun™ Sample Prep Kit (Cat No. 30PRE108).

Blood samples
Whole blood can be collected in EDTA, heparin or sodium citrate. Blood anticoagulants have a substantial consequence on PCR reactions therefore it is imperative that the optimal volume of blood is collected as indicated on the tube.

Urine Sediment
Two drops (100 µl) of 10X PBS (provided) should be added to 1 ml of fresh urine. The sample should then be cetrifuged at high speed (10,000 x rpm) for ten minutes. The supernatant is discarded and the solid material (pellet) found at the bottom of the test tube should be resuspended in one drop (50 µl) of 1X PBS (provided) prior to DNA extraction. The concentrated urine samples should be stored frozen.

For best results, recently acquired samples and freshly prepared DNA extracts are recommended for use with the PCRun™ Kit. It is recommended to process samples close to collection time. Blood samples and urine pellets must be maintained frozen at -20^{0}C if not processed immediately.
Transportation of samples should always be under cold conditions.

PROTOCOL - PCRun™ REACTION

1. Prepare a clean working area for the assay.
Working area should be decontaminated with commercial household bleach (3.5%) diluted 1:10 with water.
2. Prepare all parts of the assay:

√ **Extracted DNA sample**
√ Pouch with reaction tubes
√ Capillary tubes for dispensing 20 µl volume
√ Fine tipped permanent marker

3. Switch on the heat block and adjust to 60°C. Once the block has reached the target temperature, continue with the reaction.

4. Remove the PCRun™ strip from its protective pouch. Eight individual reaction tubes are connected by a thin plastic spacer. Employing a small clean scissors, disconnect the required number of tubes without disturbing the lids. Tap the tubes lightly on a surface and observe that the small white pellet is located on the bottom of the tube.

5. Label the lid of the tubes clearly for sample identification.

6. Carefully open the lid of the reaction tubes, one at a time. Employing the 20 µl disposable capillary tube, dispense 20 µl of DNA extracted with PCRun™ Sample Prep Kit into the reaction tube. Make sure that the entire content of the capillary tube has been emptied into the PCRun™ reaction tube. Tap the tube on a surface to bring all the fluid to the bottom of the tube. Incubate the mixture at room temperature for 1 minute to allow the pellet to completely dissolve.

7. Place the reaction tube into the appropriate hole in the pre heated block (60°C) and incubate for exactly 1 hour. Do not open the tube cover during or at the end of the incubation period.

8. At the end of the incubation period (1 hr) remove the tube from the heat block and analyze immediately with the disposable nucleic acid detection device.

ANALYSIS OF PCRun™ REACTION WITH THE DISPOSABLE NUCLEIC ACID DETECTION DEVICE

One disposable nucleic acid detection device is needed for each test. Open and remove the components of the detection device. The device consists of two plastic parts, the Amplicon Cartridge containing a plastic buffer bulb and the Detection Chamber containing the lateral flow strip (Figure 1).

1. Verify the presence of fluid in the bulb.

2. Mark each chamber with the sample ID.

3. Align the lid section of the PCRun™ reaction tube with the wide partition located beside the buffer bulb. Apply light pressure to attach the reaction tube to the Amplicon Cartridge (Figure 1).

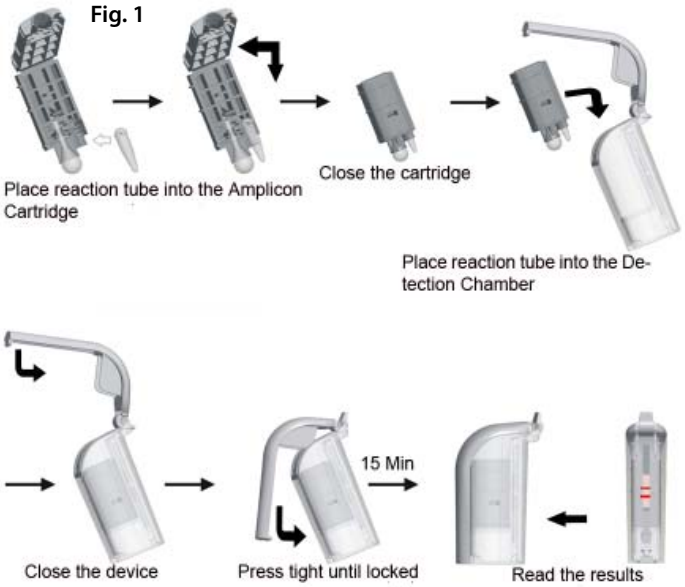
4. Fold the Amplicon Cartridge in two and snap closed. Place the cartridge into the Detection Chamber with the bulb facing downwards and away from the chamber lever.

5. Push the lever downwards to lock the device.

6. Wait for 15-30 minutes to read the results. Results read after 30 minutes are invalid.

Manufacturer: Biogal Galed Labs. Acs. Ltd.
TEL: +972 (0)4 9898605 FAX: +972 (0)4 9898690
email: info@biogal.co.il www.biogal.co.il
Kibbutz Galed, 1924000 - Israel

Fig. 1

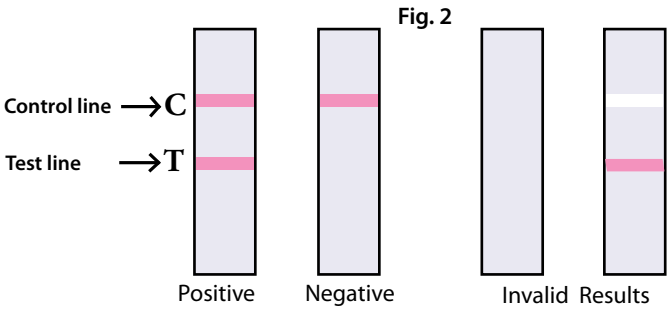


READING AND INTERPRETING THE RESULTS

A valid test must present a red control band. The control line must appear regardless of a positive or negative result. (Figure 2):

1. **Positive Result** - two bands appear , the upper control line and the lower test line. The appearance of both control line and test line indicates the presence of Leptospira pathogens.
2. **Negative Result** – a single control line appears. The appearance of a control line only, indicates the absence of the Leptospira pathogen or that the copy number is below the detection limit.

Fig. 2



LIMITATIONS

As with any diagnostic test, results acquired with the PCRun™ Molecular Detection Kit should be interpreted in consideration of all clinical and laboratory findings.
Animals undergoing antibiotic treatment will most likely display a negative PCR result.

ANALYTICAL SENSITIVITY

The PCRun™ reaction can detect a minimum of 10^2 copies of the target gene in pure DNA.

REFERENCES

1. Levett, P. N. 2001. Leptospirosis. Clin. Microbiol. Rev. 14:296–326.

2. Haake DA, Chao G, Zuerner RL, Barnett JK, Barnett D, Mazel M, Matsunaga J, Levett PN, Bolin CA (2000) The leptospiral major outer membrane protein LipL32 is a lipoprotein expressed during mammalian infection. Infect Immun 68(4):2276–2285