Canine Leishmania Molecular Detection Kit

Cat. No.30CLI116/30CLI148

For *in vitro* veterinarian diagnostic use only

User Manual

INTENDED USE

PCRun® Canine Leishmania Molecular Detection Kit is intended for detection of *Leishmania infantum* in **DNA** isolated from canine **whole blood** and **bone marrow**. 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 kinetoplastid DNA associated with *L. infantum*. It is intended for the qualitative detection of *L. infantum*. This kit is designed to be used with a compatible PCRun® Reader.

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 pac kage 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 and seal the zip-lock tightly.**
- 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

Leishmaniasis is an important vector-borne disease resulting from a protozoan infection caused by parasites classified in the family *Trypanosomatidae*. *Leishmania* spp. are usually transmitted indirectly between hosts by sandflies which act as biological vectors. Mammals can be infected asymptomatically for long periods, and they often remain chronically infected even after clinical cure. Subclinically infected animals can transmit *Leishmania* to sandflies and the parasites can also be transmitted via blood transfusions and transplacental transmission.

L. infantum is often classified as a visceral disease in humans, however dogs usually have both visceral and cutaneous involvement. Canids have been reported to be the major reservoir hosts for *L. infantum*, and dogs are the most important species in maintaining this parasite in domestic cycles. The reported incubation period for *L. infantum* in dogs varies from three months to seven years. In some dogs, severe clinical signs occur soon after the animal becomes infected while other dogs remain asymptomatic, in some cases for a lifetime. These animals can demonstrate symptoms at any time, particularly if they become immunosuppressed. The disease is usually slowly progressive.

DIAGNOSIS

Leishmaniasis may be manifested as a subclinical infection, self-limiting disease or the classical non-self-limiting severe illness. The main clinical findings associated with typical canine leishmaniasis are skin lesions. Variable symptoms such as lethargy, anorexia, weight loss, anemia, splenomegaly and local or general lymphadenopathy can accompany such an infection. Chronic renal disease, resulting in death, is common with *L. infantum* infections. Erosive or nonerosive polyarthritis with polymyositis can lead to progressive muscle atrophy[3].
Leishmaniasis can be diagnosed[3] by direct microscopic observation of amastogotes in stained macrophage present in blood, ocular granulomas, tissue collected from lymph node and bone marrow aspirates as well as skin scraping from lesions. Parasites are sometimes undetectable by this method due to low numbers. *Leishmania* spp. can be cultured in a variety of media however in vitro culture requires 5-30 days before diagnosis can be determined. Serological testing is available in the form of Indirect Fluorescent Antibody Test (IFAT), Enzyme-Linked Immunosorbent Assay (ELISA) and various agglutination methods. Most symptomatically infected dogs are seropositive, however not all asymptomatically infected animals or those with localized skin lesions have detectable antibody titers. Polymerase chain reaction (PCR) has been shown to be more sensitive than conventional techniques of microscopy and culture used alone or in combination [1,2]. PCRun® is a sensitive method to detect *Leishmania*.

KIT CONTENTS

Components	16 Test Kit	48 Test Kit
PCRun® strip of 8 lyophilized *Leishmania* single reaction tubes	2	6
PCRun® buffer to re-dissolve lyophilized reaction pellets	2 Vials, 200 µl	6 Vials, 200 µl
PCRun® lyophilized *Leishmania* Positive Control	1 vial	1 vial
Buffer to reconstitute and dilute positive control.	1 vial, 800 µl	1 vial, 800 µl

EQUIPMENT TO BE SUPPLIED BY USER:

- DNA extraction kit suitable for use with PCR reactions
- PCRun® Reader aquired from Biogal
- Timer
- Small scissors
- Fine tipped permanent marker
- Protective laboratory gloves
- Accurate laboratory pipettes with aerosol barrier tips

SAMPLE COLLECTION, STORAGE AND TRANSPORT

The kit is suitable for detecting nucleic acid extracted from whole blood and bone marrow employing most DNA extraction kits designed for use with PCR. Samples 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.
For best results, recently acquired samples and freshly prepared DNA extracts are recommended for use with the PCRun® kit.
Unless otherwise recommended, samples and DNA extracts can be maintained at 2-8^{0}C for up to 24 hours or -20^{0}C for an extended period of time.

PROTOCOL - PCRUN® REACTION

1. Prepare a clean working area for the assay. Work area should be 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
- √ PCRun® buffer
- √ Pipettors for dispensing 5, 15 and 500 µl volume
- √ PCRun® Positive Control
- √ Positive Control Dilution Buffer
- √ Fine tipped permanent marker
- √ PCRun® Reader (Please refer to the PCRun® Reader Instruction Manual for operating directions)

3. **Positive Control**
A positive control is supplied with the kit. It is recommended that a positive control be run at the same time as the PCRun® reactions.

Dilution to final concentration of 10^6 copies/5 µl .

a. Add 500 µl Positive Control Dilution Buffer to the vial containing the lyophilized pellet. Vortex the vial and allow to stand 5 min at room temperature. Vortex again. The vial contains 10^6 copies of the target gene/5µl. Label the tube with the concentration. This dilution will be employed as the positive control.
b. Use 5 µl of the positive control in place of the DNA sample for PCRun® positive control reactions. It is not advisable to repeatedly freeze and defrost the Positive Control. The remainder of solution should be aliquoted into small volumes and maintained at -20^0 C for later use.

The positive control can be a source of contamination therefore maximum attention must be applied to ensure that the positive control does not come in contact with any other kit components. The positive control should be added to the reaction tube following completion of the test samples.

4. Switch on the PCRun® Reader and note that it is adjusted to 60^{0}C. Once the PCRun® Reader has reached the target temperature, continue with the reaction.

5. Remove the PCRun® strip from its protective pouch. Take care to return the unused tubes to the aluminium envelope and seal completely with zip-lock to maintain a dry environment. 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.

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

7. Carefully open the lid of the reaction tubes, one at a time. Dispense 15 µl of PCRun® Buffer to each reaction tube. Close the lid and incubate the mixture at room temperature for 1 minute to allow the pellet to completely dissolve.

8. Add 5 µl of DNA sample into the PCRun® reaction tube and mix thoroughly. Close the lid of the tube firmly and tap the tube on a surface to bring all the fluid to the bottom of the tube.

9. Place the reaction tube into the PCRun® Reader which has been pre heated to 60°C and incubate for exactly 1 hour. Do not open the tube cover during or at the end of the incubation period.

ANALYSIS OF PCRUN® REACTION

After one hour incubation, final results of each reaction will appear on the touch screen. Follow instructions found in the manual accompanying the PCRun® Reader.

LIMITATIONS

As with any diagnostic kit, the results obtained must be used as an adjunct to other clinical and laboratory findings. The accuracy of the test results depends on the quality of the sample and adherence to protocol. A negative result may be obtained if the specimen is inadequate or target pathogen concentration is below the sensitivity of the test.
Animals undergoing treatment will most likely display a negative PCR result.

ANALYTICAL SENSITIVITY

The PCRun® reaction can detect 10^3 copies of the target gene in pure DNA.

REFERENCES

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