Canine Leishmania Molecular Detection Kit

Cat. No.30CLI108

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 *Leishmania infantum*. This kit is designed to be used with a compatible heat block.

STORAGE AND HANDLING

- Storage 2-25°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

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 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	Contents	Amount
Aluminum pouch **Cat No. 03CLI100**	PCRun™ strip of 8 lyophilized *Leishmania* single reaction tubes	1
Detection device **Cat No. 03100010**	Aluminium pouch with disposable nucleic acid detection device.	8
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 pipettes.**

EQUIPMENT TO BE SUPPLIED BY USER:

- Biogal PCRun™ Sample Prep
- Heat block which maintains 60°C – compatible with 0.2 PCR tubes. Heat block can be supplied by Biogal
- Timer
- Small scissors
- Fine tipped permanent marker
- Protective laboratory gloves

SAMPLE COLLECTION, STORAGE AND TRANSPORT

The kit is suitable for detecting nucleic acid extracted from 50 µl of whole blood and bone marrow using PCRun™ Sample Prep Kit (Cat No. 30PRE108). 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 optimal 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°C for up to 24 hours or -20°C for an extended period of time.

PROTOCOL - PCRun™ REACTION

1. Prepare a clean working area for the assay. Work 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 60⁰C. Once the block has reached the target temperature, continue with the reaction.

4. Remove the PCRun™ strip from its protective pouch. Take care to return the unused tubes to the aluminium envelope and seal completely with tape 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.

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.

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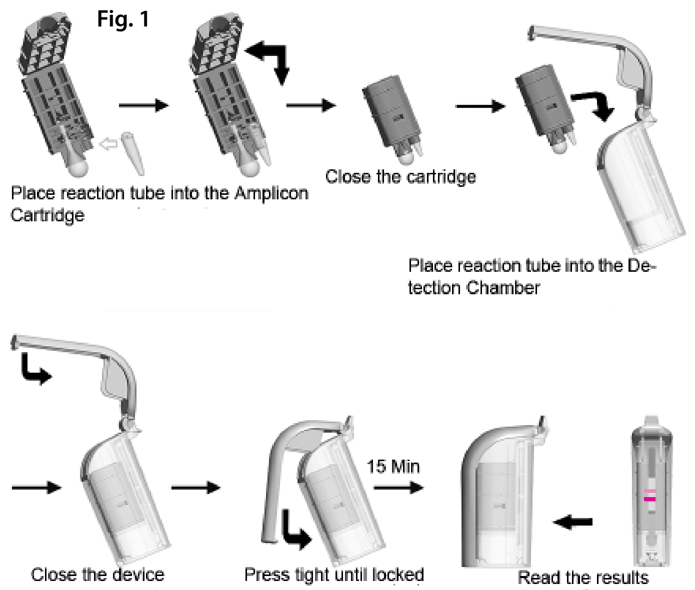
Fig. 1

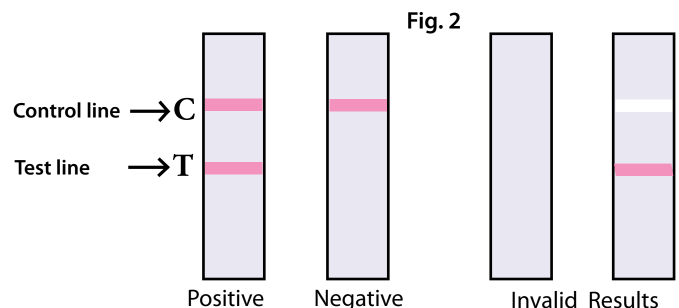


READING AND INTERPRETING THE RESULTS

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 *Leishmania infantum*.
2. **Negative Result** – a single control line appears. The appearance of a control line only, indicates the absence of the *Leishmania infantum* DNA or the that the copy number is below the detection limit.

Fig. 2



LIMITATIONS

As with any diagnostic test, results of the PCRun™ molecular 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 10^2 copies of the target gene in pure DNA.

REFERENCES

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