

Canine Ehrlichia Molecular Detection Kit

Cat. No.30CEH108

For *in vitro* veterinarian diagnostic use only

User Manual

INTENDED USE

PCRrun™ Canine Ehrlichia Molecular Detection Kit is intended for detection of *Ehrlichia canis* in **DNA** isolated from canine **whole blood** and **bone marrow**. The kit can be used for detection of acute infections. It contains all the disposable components required for performing an easy and accurate test.

PRINCIPLE

PCRrun™ is a molecular assay based on isothermal amplification of part of the 16s rDNA gene. It is intended for the qualitative detection of *Ehrlichia canis*. 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 PCRrun™ 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 PCRrun™ reaction tubes from the sealed pouches only immediately prior to their use.
- **Return unused PCRrun™ 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

Ehrlichia is a Gram-negative coccobacillus obligate intracellular bacterium with tropism for hematopoietic cells. Canine monocytic ehrlichiosis (CME) is primarily caused by *E. canis* and its principle vector is the tick, *Rhipicephalus sanguineus*⁽¹⁾. CME is a potentially fatal disease that requires early diagnosis in order to initiate the correct therapy necessary for a good prognosis.

Ehrlichiosis is characterized by an incubation period of 8 to 20 days, followed by acute, subclinical and in some cases, chronic phases. Thrombocytopenia is considered to be the most common and consistent hematological abnormality of dogs infected with *E. canis*, but the disease can display a wide variety of clinical signs such as depression, lethargy, weight loss, anorexia, pyrexia, lymphadenomegaly, splenomegaly and hemorrhagic diathesis. Thrombocytopenia, mild anemia and mild leukopenia are apparent during the acute stage; mild thrombocytopenia in the subclinical stage; and pancytopenia in the severe chronic stage. Hypoalbuminemia,

hyperglobulinemia, and hypergammaglobulinemia are the main biochemical abnormalities⁽²⁾.

Dogs with mild clinical signs or in the acute stage of illness show dramatic improvement in clinical and hematologic parameters within 24 to 48 h after therapy is instituted, while dogs with chronic ehrlichiosis have a poor prognosis.

DIAGNOSIS

A combination of lab tests along with clinical signs and history are used to make a diagnosis. Diagnostic tests include microscopic examination of blood smears, indirect fluorescent antibody (IFA), enzyme-linked immunosorbent assay, ELISA ImmunoComb and molecular analysis such as polymerase chain reaction (PCR). Experience is required to correctly microscopically identify the presence of morulae in blood monocytes. The pathogen inclusions are transient, but if observed, a diagnosis can be confirmed. Serum antibodies have been detected as early as 7 days after initial infection, but some dogs may not become seropositive until 28 days after infection. Clinical signs of disease usually occur before the development of serum antibodies and in some cases when acutely infected dogs are immunosuppressed, antibody test results can be negative. Serum titers reflect the quantity of antibodies present in a serum sample; however, titers do not always correlate with the duration of infection or the severity of disease. It must be noted that elevated antibodies to *Ehrlichia* is a strong indication of exposure to the pathogen. PCRrun™ molecular analysis can detect clinical disease before seroconversion. It is the consensus of the American College of Veterinary Internal Medicine that molecular analysis should be used in conjunction with serology for the initial diagnosis of ehrlichiosis in untreated animals. In addition, PCR should be employed to monitor the stages of treatment and post antimicrobial therapy.⁽³⁾

KIT CONTENTS

Components	Contents	Amount
Aluminum pouch Cat No. 03CEH100	PCRrun™ strip of 8 lyophilized <i>Ehrlichia</i> 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 PCRrun™ 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 PCRrun™ 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 best results, recently acquired samples and freshly prepared DNA extracts are recommended for use with the PCRrun™ 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.

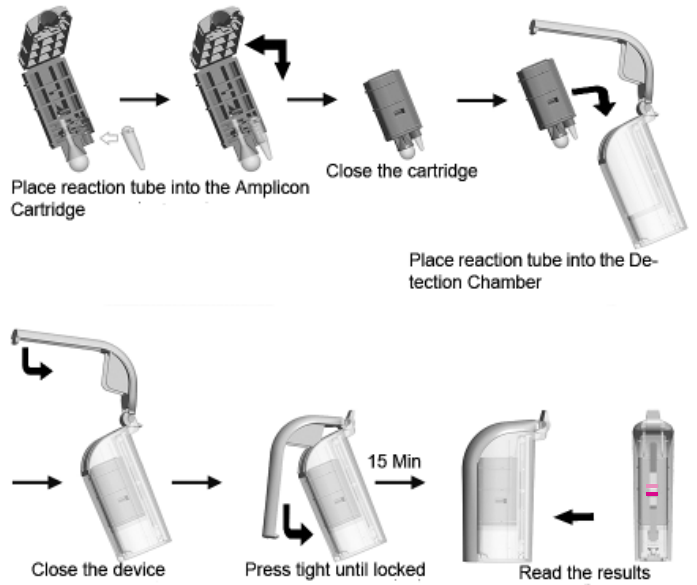
PROTOCOL - PCR[™] REACTION

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 60°C. Once the block has reached the target temperature, continue with the reaction.
4. Remove the PCR[™] 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 PCR[™] Sample Prep kit into the reaction tube. Make sure that the entire content of the capillary tube has been emptied into the PCR[™] 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 PCR[™] 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).

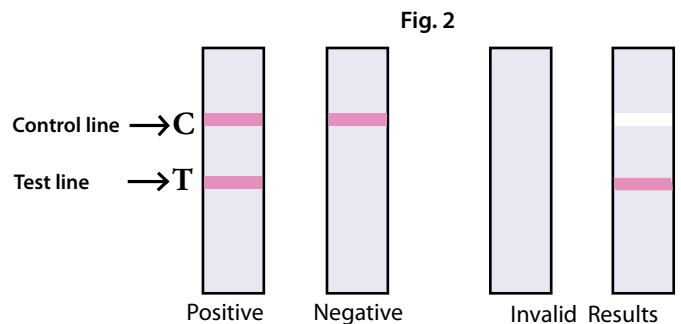
1. Verify the presence of fluid in the bulb.
2. Mark each chamber with the sample ID.
3. Align the lid section of the PCR[™] reaction tube with the wide partition located beside the buffer bulb. Apply light pressure to attach the reaction tube to the Amplicon Cartridge (Figure 1).
4. Fold the Amplicon 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.



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



LIMITATIONS

As with any diagnostic test, results of the PCR[™] 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 PCR[™] reaction can detect 10² copies of the target gene in pure DNA.

REFERENCES

1. Human *Ehrlichiosis* and *Anaplasmosis*.
Ismail, Nahed, Karen C. Bloch, and Jere W. McBride.
Clinics in Laboratory Medicine 30.1 (2010): 261-92
2. Demonstration of serum antiplatelet antibodies in experimental acute canine *Ehrlichiosis*.
Waner T., Harrus S., Weiss D. J., Bark H., Keysary A. (1995)
Vet. Immunol. Immunopathol. 48:177-182.
3. Consensus Statement on Ehrlichial Disease of Small Animals from the Infectious Disease Study Group of the ACVIM
T. Mark Neer, Edward B. Breitschwerdt, Russell T. Greene, and Michael R. Lappin. J Vet Intern Med 2002;16:309-315



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