NEW

PCRun®

Canine *Babesia canis* Molecular Detection Kit

Cat. No.30CBC104

For *in vitro* veterinarian diagnostic use only

User Manual

INTENDED USE

PCRun® Canine *Babesia canis* Molecular Detection Kit is intended for detection of *Babesia canis canis* and *Babesia canis vogeli* in **DNA** isolated from canine **whole blood**. The kit should be used for detection of acute infections. It 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 18s rDNA gene. It is intended for the qualitative detection of *B. canis* and *B. vogeli*. 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 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

Canine babesiosis is a worldwide, tick-borne, protozoal disease caused by haemoparasites of the genus Babesia. The two predominant species known to naturally infect dogs are *B. canis* and *B. gibsoni*. *B. canis* is a large piriform-shaped organism that exists singly or paired within erythrocytes while *B. gibsoni* is a small pleomorphic organism. *B. canis* has been differentiated into three sub-species (*B. canis canis, B. canis vogeli*, and *B. canis rossi*) on the basis of cross-immunity, serological testing, vector specificity and molecular phylogeny. The infection is more prevalent in seasons and geographical regions with high prevalence of ticks and other arthropod vectors. Transmission is also possible through blood transfusion or blood-contaminated fomites. The incubation period between exposure to the parasite and symptoms is on average two weeks. Symptoms may be intermittent and can include lack of energy, anorexia, weakness, fever, pale gums, orange or red-colored urine, discolored stool and enlarged lymph nodes. Intracellular replication of babesia parasites results in both direct and immune-mediated hemolytic anemia. Thrombocytopenia is a hallmark of the disease, but petechiation or epistaxis is very rarely seen, except in cases with concomitant Ehrlichia or Theileria infections. A severe infection can affect multiple organ systems including the lungs, GI tract, kidneys, and nervous system. Severity of symptoms depends on the species of parasite involved and on the ability of the dog's immune system to defend against it. Dogs which have survived babesiosis often remain sub-clinically infected and may suffer relapse or serve as a source for further spread of the disease.

DIAGNOSIS

Babesiosis is typically diagnosed in the acute phase by identifying the organism in Wright's or Giemsa stained blood smears. Diagnosis of chronically infected and carrier dogs is difficult due to very low, often intermittent parasitaemias. Indirect Fluorescent Antibody (IFA) and ELISA tests can retrospectively determine disease by detecting antibodies which may require up to 10 days to reach the detection limit. Although clinical disease may resolve, Babesia infections are often persistent in dogs. Even after appropriate therapy, infection can persist for the life of the dog.
Polymerase Chain Reaction (PCR) offers a highly sensitive alternative to blood smear examination for diagnosis during active disease and can detect clinical disease before seroconversion. In addition PCR may also be of use for detection of persistent infection, which may last up to 27 months.

KIT CONTENTS

Components	Amount
PCRun® strip of 4 lyophilized *Babesia* single reaction tubes	1
Aluminium pouch with disposable nucleic acid detection device.	4
Disposable plastic capillary tubes 20 µl*	5

***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 using PCRun® Sample Prep Rapid DNA Extraction Kit (Cat No. 30PRE104). 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°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 60°C. 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 zip-lock to maintain a dry environment. Four 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

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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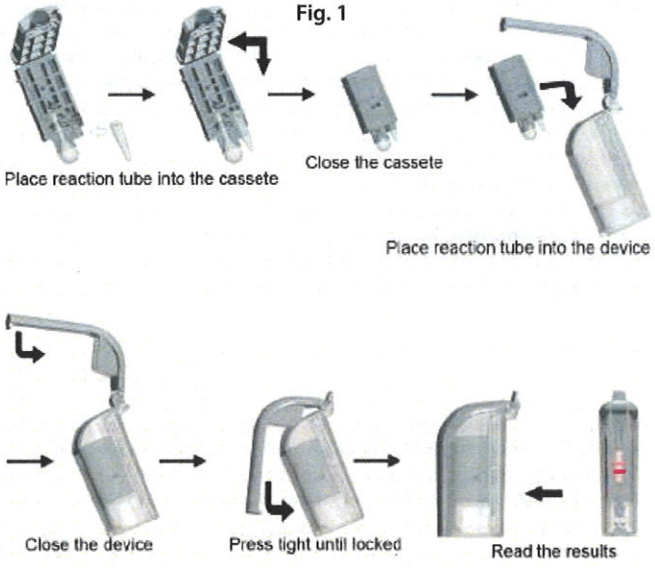
Fig. 1

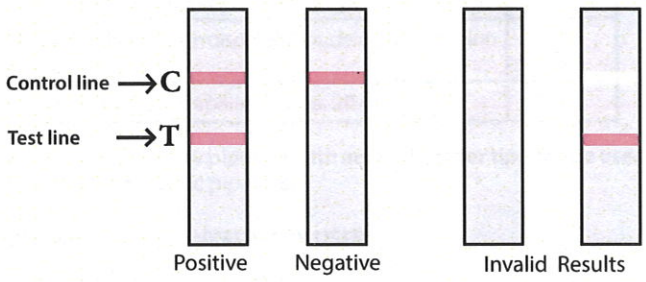


READING AND INTERPRETING THE RESULTS

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

Fig. 2



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 with anti-babesian drugs 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

1. Babesia – A historical overview, Gerrit Uilenberg. Veterinary Parasitology, 138 (2006) 3-10.
2. Canine babesiosis: from molecular taxonomy to control, Peter J Irwin. Parasites and Vectors (2009), 2 Suppl1):S4.
3. Canine babesiosis – a never-ending story, Friederike Krämer. CVBD Digest No.4 July 2009.