Canine *Babesia canis* Molecular Detection Kit

Cat. No.30CBC116/30CBC148

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 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 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. Fighting between dogs is also thought to be a likely mode of mechanical transmission.

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	16 Test Kit	48 Test Kit
PCRun® strip of 8 lyophilized *Babesia canis* single reaction tubes	2	6
PCRun® buffer to re-dissolve lyophilized reaction pellets	2 Vials, 200 µl	6 Vials, 200 µl
PCRun® lyophilized *Babesia canis* 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 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 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 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

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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.

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