PCRun®

Canine *Babesia Species* *(B. canis, B. vogeli, B. rossi, B. gibsoni, B. negvi)*

Molecular Detection Kit

Cat. No.30CBS116/30CBS148

For *in vitro* veterinarian diagnostic use only

User Manual

INTENDED USE

PCRun® Canine *Babesia Species* Molecular Detection Kit is intended for detection of *B. canis, B. vogeli, B. rossi, B. gibsoni, B. negvi* in **DNA** isolated from canine **whole blood**. 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, B. vogeli, B. rossi, B. gibsoni, B. negvi*. This kit is designed to be used with a compatible PCRun® Reader.

SPECIFICATIONS

Specimen	DNA
Test time	10 minutes hands on 75 minutes total
Specimen volume	5µl
Sensitivity	98.8%
Specificity	97.5%
Storage temperature	2-25 °C /36-86 °

ANALYTICAL SENSITIVITY

The PCRun® reaction can detect 10^3/5µl copies of the target gene in pure DNA.

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 pouch 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, a tick transmitted disease of dogs, is caused by a variety of intra erythrocytic protozoal parasites of the genus *Babesia*. Two microscopic forms[1] have been distinguished; large oval or pear shaped singular or paired piroplasms (subspecies *B. canis, B. vogeli, and B. rossi*) and small organisms with single to multiple signet ring (*B. gibsoni, B. negvi and B. vulpes* (syn. *B. micorti*-like piroplasm or *Theileria annae), B. annae and B. microti* [1,2,3]).

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 (*B. gibsoni)*.

The subspecies have variable virulence and durations of incubation (1-3 weeks), with clinical signs ranging from mild to severe.

DIAGNOSIS

Diagnostic tests include direct parasite detection by Giemsa or fluorescent antibody staining of blood smears, indirect immunofluorescent antibody test and polymerase chain reaction. Microscopic analysis requires advanced skills since parasitemia can be low. A positive serological test relies on an antibody response, which may take up to ten days to develop. Once antibodies are generated, they may persist for years making it difficult to differentiate between a present and past infection. **PCR, in addition to providing a high level of sensitivity, has the advantage in that it can be designed to detect, all the relevant canine Babesia species suited for broad screening of canines presumed to be infected with Babesia.**

KIT CONTENTS

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

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 500µl 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/5µl .

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.

REFERENCES

1. Overview of Canine Babesiosis, Poonam Vishwakarma and M.K. Nandini (2019). Veterinary Medicine and Pharmaceuticals, IntechOpen. Chapter 1.
2. A new piroplasmid species infecting dogs: morphological and molecular characterization and pathogeny of *Babesia negevi n. sp.*. Gad Beneth et al (2020). "https://parasitesandvectors.biomedcentral.com/"Parasites & Vectors, volume 13, Article number: 130
3. Establishment of *Babesia vulpes n. sp.* (*Apicomplexa: Babesiidae*), a piroplasmid species pathogenic for domestic dogs. Gad Beneth et al (2019). "https://parasitesandvectors.biomedcentral.com/"Parasites & Vectors, volume 12, Article number: 129.

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