PCRun®

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

Molecular Detection Kit

Cat. No.30CBS104

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 heat block.

SPECIFICATIONS

Specimen	**DNA**
Test time	**10 minutes hands on 75 minutes total**
Specimen volume	**20µl**
Sensitivity	**98.8%**
Specificity	**97.5%**
Storage temperature	**2-25°C / 36-86°F**

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 (refrigerated or room temperature).
- 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 pouch 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]). Babesiosis is primarily spread by infected tick species. 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	Amount
PCRun® strip of 4 lyophilized *Babesia spp.* single reaction tubes	1
Aluminium pouch with disposable nucleic acid detection device.	4
Disposable plastic capillary tubes 20µl*	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^{0}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 50µl of whole blood using PCRun® Sample Prep 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^{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
√ Capillary tubes for dispensing 20µl 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 pouch 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 20µl disposable capillary tube, dispense 20µl 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 minutes to read the results. Results read after 30 minutes are invalid.

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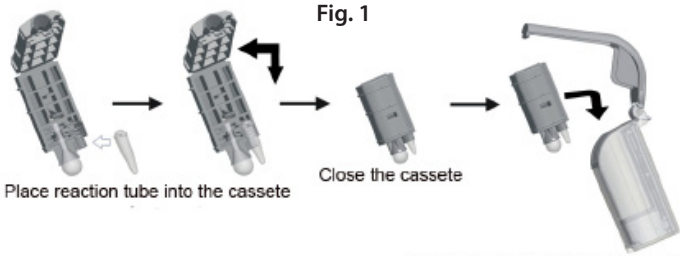
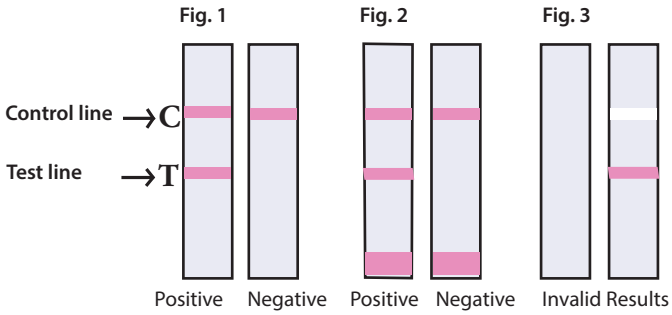
Fig. 1



READING AND INTERPRETING THE RESULTS

A valid test must present a red control band. Only the red lines of C & T apply to the test results (Figure 1). Any additional red markings along the base of the lateral strip should be ignored (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 spp*.
2. **Negative Result** – a single control line appears. The appearance of a control line only, indicates the absence of the *Babesia spp.* DNA or that the copy number is below the detection limit.



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.