PCRun®

Canine *Babesia gibsoni* Molecular Detection Kit

Cat. No.30CBG108

For *in vitro* veterinarian diagnostic use only

User Manual

INTENDED USE

PCRun® Canine *Babesia gibsoni* Molecular Detection Kit is intended for detection of *Babesia gibsoni* in **DNA** isolated from canine **whole blood**. 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

PCRun® Canine *Babesia gibsoni* kit is a molecular assay based on isothermal amplification of part of the mitochondrial protein-coding gene, Cytochrome B oxidase (cob). It is intended for the qualitative detection of *Babesia gibsoni*. The kit is designed to be used with a compatible heat block **.**

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

Babesia gibsoni is an intraerythrocytic apicomplexan parasite that causes piroplasmosis in dogs. The organisms are pleomorphic and appear most commonly within erythrocytes as individual ring forms or piriform bodies. The disease is transmitted naturally by ticks, but transmission by dog bites, blood transfusions as well as transmission via the transplacental route to the developing fetus have been reported. Infections with *B. gibsoni* have been identified worldwide, and it is now recognized as a serious emergent disease in small animal medicine. The parasite is considered endemic in Asia, Africa, the Middle East, North America and Australia.[1,2]

Clinical manifestations are variable and are mainly characterized by remittent fever, progressive anemia, thrombocytopenia, marked splenomegaly, hepatomegaly, and in some cases, death. The incubation period varies between 2-40 days depending on the route of infection and number of parasites in the inoculum. Most recovered dogs develop a state of premunition that is a balance between the host's immune response and the parasite's ability to induce clinical disease. In this state, dogs are at risk of recrudescence. Treatment is not effective in eliminating the parasite and recovered dogs commonly become chronic carriers, becoming a source for transmission of the disease via ticks to other animals. To effectively control the spread of *B. gibsoni* infections, rapid, accurate diagnosis followed by prompt effective treatment and the prevention of chronic carriers are imperative.[3]

DIAGNOSIS

The simplest and most accessible diagnostic tool is the compilation of diagnostic symptoms and microscopic examination of Giemsa or Wright's-stained capillary blood smears during acute infection. However, the diagnosis of chronically infected and carrier dogs remains a significant challenge due to very low and often intermittent parasitemia. The Immunofluorescence Antibody Assay (IFA) Test can be used to detect dogs with occult Babesiosis but antibodies employed in this test display cross reactivity between *B. canis* and *B. gibsoni*. Similar difficulties exist with ELISA testing. During primary infections, dogs with acute Babesiosis can be serologically negative, necessitating repeated testing using convalescent sera. Treatment choices are largely dependent on the Babesia species identified therefore accurate classification is necessary. Individual Babesia species have historically been characterized by size and morphological appearance of the intra-erythrocytic forms therefore further differentiation of the species based on parasite recognition using blood smears is required. Parasite morphology by microscopic examination is prone to subjective error, but species specific polymerase chain reaction (PCR) provides an alternative rapid diagnostic test with good sensitivity and specificity when blood samples are collected early in the course of the clinical disease, and prior to initiation of chemotherapy.[4,5,6]

KIT CONTENTS

Components	Contents	Amount
Aluminum pouch **Cat No. 03CBG100**	PCRun® strip of 8 lyophilized *Babesia gibsoni* 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 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 50 µl of whole blood using PCRun® 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 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 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. 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 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.

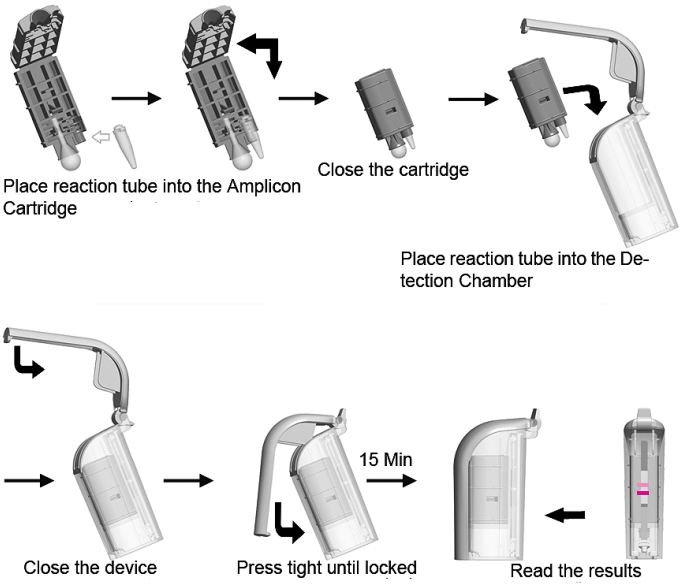
8. At the end of the incubation period (1 hr) remove the tube from the heat block and analyze immediately with the disposable nucleic acid detection device.

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 15-30 minutes to read the results. Results read after 30 minutes are invalid.

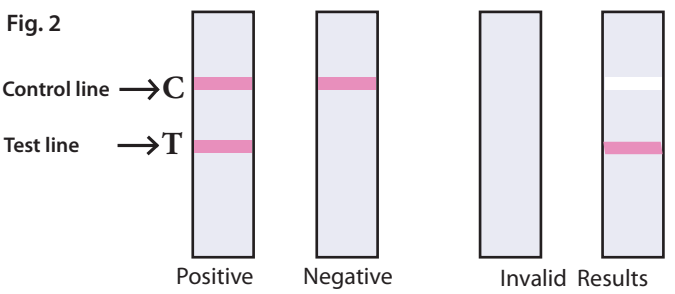
Manufacturer: Biogal Galed Labs. Acs. Ltd.
TEL: +972 (0)4 9898605 FAX: +972 (0)4 9898690
email: info@biogal.co.il www.biogal.co.il
Kibbutz Galed, 1924000 - Israel



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 gibsoni*.
2. **Negative Result** – a single control line appears. The appearance of a control line only, indicates the absence of the *Babesia gibsoni* 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.

ANALYTICAL SENSITIVITY

The PCRun® reaction can detect 10^2 copies of the target gene in pure DNA.

REFERENCES

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4- **Clinical, haematological, cytokine and acute phase protein changes during experimental Babesia gibsoni infection of beagle puppies. Brown AL, Shiel RE, Irwin PJ. Exp Parasitol. 2015 Oct;157:185-96.**

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6- **Optimization of a molecular method for the diagnosis of canine babesiosis. Moraes PH, Rufino CP, Reis T, Aguiar DC, Meneses AM, Gonçalves EC. Rev Bras Parasitol Vet. 2014 Mar;23(1):105-8**