PCRun® QUATTRO

Tick-Borne Molecular Detection Panel

Cat. No.30QTR102

For *in vitro* veterinarian diagnostic use only

User Manual

INTENDED USE

PCRun® Quattro Tick-Borne Molecular Detection Panel is intended for the detection of four tick-borne pathogens: *Ehrlichia canis*, *Anaplasma platys*, *Babesia canis* (*canis and vogeli)* and *Babesia gibsoni* in DNA isolated from whole blood. The kit has been designed to be used for detection of acute infections and contains all the disposable components required for performing an easy and accurate test for these four pathogens.

PRINCIPLE

PCRun® Quattro is a molecular assay based on isothermal amplification of pathogen specific genes. It is intended for the qualitative detection of four separate tick-borne organisms, *E. canis, A. platys, B. canis (canis and vogeli) and B. gibsoni*. This 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

Tick-borne diseases are transmitted by means of the bite of an infected tick. Ticks are known to be carriers of a variety of potential pathogenic organisms such as rickettsia, bacteria, virus and protozoa. An individual tick can harbor multiple agents and as a result co-infections can occur, compounding the difficulty in diagnosis and treatment. The diagnosis and treatment of infections resulting from *E. canis, A. platys, B. canis and B. gibsoni* can prove to be problematic due to the similarity in clinical signs and laboratory findings.

DIAGNOSIS

Clinical features, history and laboratory tests are imperative for accurate diagnosis of the disease. Historically, microscopic examination of blood smears have become the most common test used but correct identification of the target pathogen is subjectively difficult with a high occurrence of incorrect calls. Additional tests include Indirect Fluorescent Antibody (IFA), Enzyme-Linked Immunosorbent Assay (ELISA) and molecular analysis such as Polymerase Chain Reaction (PCR). Use of antibody testing for acute stages of disease may give false negative results as clinical signs precede the generation of antibody titers and immunocompromised patients do not produce measurable antibody. The presence of antibody titers does not always define a present disease, but can be an indication of a previous exposure to the pathogen. In order to verify infection, sero-conversion or a four-fold increase in titer must be demonstrated.

PCRun® Quattro provides a rapid differential diagnostic test for all four pathogens present in this panel. The detection level of this test is highly specific and sensitive during active disease and is applicable prior to seroconversion and appearance of measurable antibody titers. The test provides an accurate aid in diagnosis allowing for an optimal treatment protocol.[1,2]

KIT CONTENTS

Components	Contents	Amount
Aluminum pouch **Cat No. 03QTR100**	Four color-coded PCR tubes. (See color coding below)	2
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

Color code:
Red - *Ehrlichia canis*, Yellow - *Anaplasma platys,* Blue - *Babesia canis*, Green - *Babesia gibsoni*

***Accurate laboratory pipettes with aerosol barrier tips can be used in place of the capillary tubes.**

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 4 PCRun® tubes from their protective pouche.
Note that each color represents a different disease:
Red - *Ehrlichia canis*, Yellow - *Anaplasma platys*, Blue - *Babesia canis*, Green - *Babesia gibsoni* .
Take care to return the unused tubes to the aluminium envelope and seal completely with zip-lock to maintain a dry environment. 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 each 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 tubes into the appropriate holes 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 tubes from the heat block and analyze immediately with the disposable nucleic acid detection devices.

ANALYSIS OF PCRun® REACTION WITH THE DISPOSABLE NUCLEIC ACID DETECTION DEVICE

One disposable nucleic acid detection device is needed for each tube. 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).

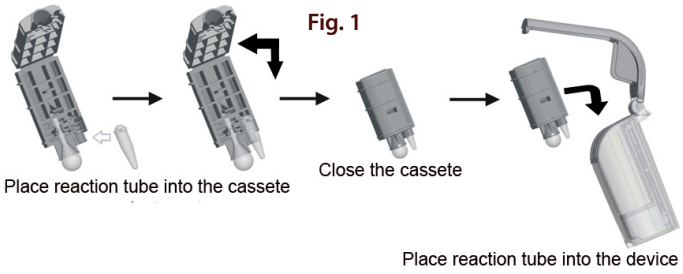
4. Fold the Amplicon Cartridge in two and snap closed. Place the cartridge into the Detection Chamber with the bulb facing downwards and away from the chamber lever.

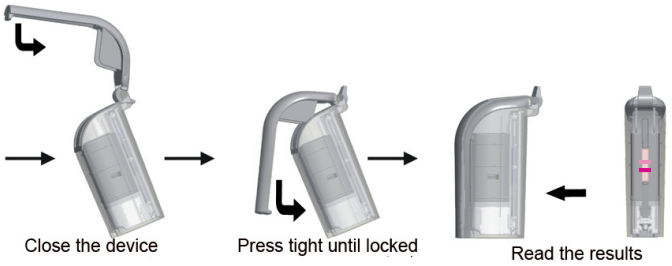
5. Push the lever downwards to lock the device.

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

Fig. 1



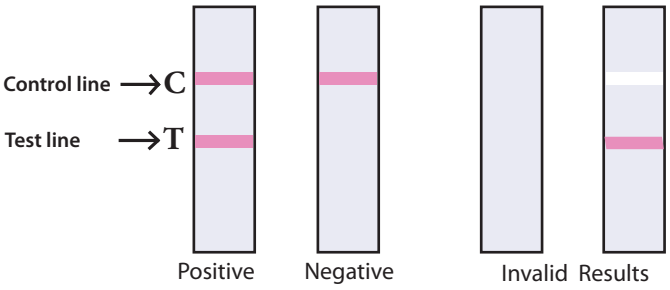


READING AND INTERPRETING THE RESULTS

Each test tube is read separately. The color codes are:
Red - *Ehrlichia canis*, Yellow - *Anaplasma platys*, Blue - *Babesia canis*, Green - *Babesia gibsoni* .
The presence of more than one positive reaction indicates co-infection.
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 the target DNA.
2. **Negative Result** – a single control line appears. The appearance of a control line only, indicates the absence the target 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 antibiotic or anti-protozoal treatment will most likely display a negative PCRun® result.

ANALYTICAL SENSITIVITY

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

REFERENCES

1. Are vector-borne pathogen co-infections complicating the clinical presentation in dogs? De Tommasi et al. Parasites & Vectors 2013, 6:97.

2. CVBD Digest No 2 2008. A challenge for the practitioner- co-infection with vector-borne pathogens in dogs.