PCRun® QUATTRO

Tick-Borne Molecular Detection Panel

Cat. No.30QTR104

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 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

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	Four color-coded PCR tubes. (See color coding below)	4
PCRun® buffer to re-dissolve lyophilized reaction pellets	4 vials	200 µl each

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

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 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 and 15 µl volume
√ Fine tipped permanent marker
√ PCRun® Reader (Please refer to the PCRun® Reader Instruction Manual for operating directions)

3. Switch on the PCRun® Reader and note that it is adjusted to the program "GENERIC". Once the PCRun® Reader has reached the target temperature (60° C), 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 lids of the tubes clearly for sample identification.

6. 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.

7. Add 5 µl of DNA sample into the PCRun® reaction tubes and mix thoroughly. Close the lids of the tubes firmly and tap the tubes on a surface to bring all the fluid to the bottom of the tubes.

8. Place the reaction tubes 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.
Each test tube is read separately. The color codes are:
Red - *Ehrlichia canis*, Yellow - *Anaplasma platys*, Blue - *Babesia canis*, Green - *Babesia gibsoni* .
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 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.

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