PCRun®

Canine Anaplasma Molecular Detection Kit

Cat. No.30CAP116/30CAP148

For *in vitro* veterinarian diagnostic use only

User Manual

INTENDED USE

PCRun® Canine Anaplasma Molecular Detection Kit is intended for detection of *Anaplasma platys* 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® is a molecular assay based on isothermal amplification of part of the Citrate Synthase (gltA) gene. It is intended for the qualitative detection of *Anaplasma platys*. This kit is designed to be used with a compatible PCRun® Reader.

STORAGE AND HANDLING

- Storage 2-25⁰C (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

Anaplasma platys (formerly known as *Ehrlichia platys*) is an obligate intracellular Gram negative bacteria allocated to the order *Rickettsiales*, family *Anaplasmataceae*[1]. The bacterium is the causative agent of Infectious Canine Cyclic Thrombocytopenia (ICCT), a disease which is assumed to be transmitted through the bite of infected ticks. Dogs are the most common mammalian host, although rare reports of infection in cats have been documented[2]. Naturally occurring infections can result in mild to severe clinical disease. *Anaplasma platys* attacks platelets, damaging the normal blood clotting system. During the initial bacteremic phase, the platelet counts are reduced and some canines will demonstrate clinical evidence of bleeding. Symptoms of this infection can include fever, depression, bleeding from the nose or mouth, pale mucous membranes, petechial hemorrhages and lymphadenopathy. Following tick transmission, dogs can remain sub-clinically infected and chronically harbor the bacteria for months without showing symptoms of disease. The infection may not become apparent until the dog's immune system is weakened by factors such as stress or additional disease conditions.

DIAGNOSIS

Hematological abnormalities in dogs with anaplasmosis include thrombocytopenia, which is the most consistent laboratory abnormality. In severe cases, anemia is also present. During bacteraemia and subsequent thrombocytopenia, platelet counts can fall below 20,000/µl. A diagnosis may be made by microscopic detection of *A. platys* morulae. When present, these inclusion bodies can be seen within platelets on Giemsa stained blood films or buffy coat smears. Due to cyclic parasitemia, which can recur at 1 to 2 week intervals, the pathogen is often absent or present in very low numbers. For this reason microscopic analysis is not reliable and can often lead to false negative results. In addition, false positive results can occur when artifacts similar to inclusion bodies are mistaken for *A. platys* morulae. Biochemical findings may include hypoalbuminemia, hyperglobulinemia, elevated plasma alkaline phosphatase or hyperbilirubinemia. Co-infection with additional canine vector-borne pathogens can occur. This state may exacerbate the disease severity and alter the clinical presentation resulting in a complicated diagnosis, treatment and prognosis.

An Indirect Fluorescent Antibody Testing (IFAT) protocol has been developed for the detection of serum antibodies to *A. platys*. Seroprevalence is high in endemic areas therefore a diagnosis cannot be based on a single positive titer (which may only reflect previous exposure). During early acute infections, antibodies may be unapparent. A minimal four-fold increase in antibody titers is essential to confirm the diagnosis. Paired serum specimens taken at least two to three or more weeks apart are imperative for evaluation (Center for Disease Control, USA). Molecular based methods such as PCRun®, can be employed for the accurate detection of *A. platys* when parasitemia is low. Properly performed PCR based assays are the most sensitive and accurate method for the detection of Anaplasmosis caused by *A. platys* during the acute and later cyclic parasitemic stages[3].

KIT CONTENTS

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

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 2-8⁰C for up to 24 hours or -20⁰C 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, 15 and 500 µl 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/5 µl .**

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 -20⁰ 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 60⁰C. 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 antibiotic treatment will most likely display a negative PCR result.

ANALYTICAL SENSITIVITY

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

REFERENCES

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