

PCRRun®

Canine Anaplasma Molecular Detection Kit

Cat. No.30CAP108

For *in vitro* veterinarian diagnostic use only

User Manual

INTENDED USE

PCRRun® 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

PCRRun® is a molecular assay based on isothermal amplification of part of the Citrate Synthase (*glcA*) gene. It is intended for the qualitative detection of *Anaplasma platys*. This kit is designed to be used with a compatible heat block.

STORAGE AND HANDLING

- Storage 2-25°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 PCRRun® 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 PCRRun® reaction tubes from the sealed pouches only immediately prior to their use.
- **Return unused PCRRun® 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 documented². 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/ μ l. 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 PCRRun®, 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 stages³.

KIT CONTENTS

Components	Contents	Amount
Aluminum pouch Cat No. 03CAP100	PCRRun® strip of 8 lyophilized <i>Anaplasma</i> 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 μ l*	10

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

EQUIPMENT TO BE SUPPLIED BY USER:

- Biogal PCRRun® Sample Prep
- Heat block which maintains 60°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 μ l of whole blood using PCRRun® 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 PCRRun® kit. Unless otherwise recommended, samples and DNA extracts can be maintained at 2-8°C for up to 24 hours or -20°C for an extended period of time.

PROTOCOL - PCR^{un}® 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 PCR^{un} 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 PCR^{un} Sample Prep kit into the reaction tube. Make sure that the entire content of the capillary tube has been emptied into the PCR^{un} 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.

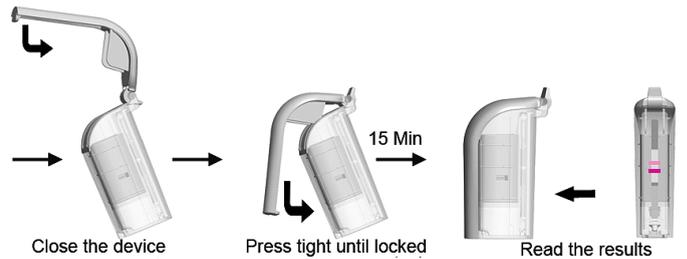
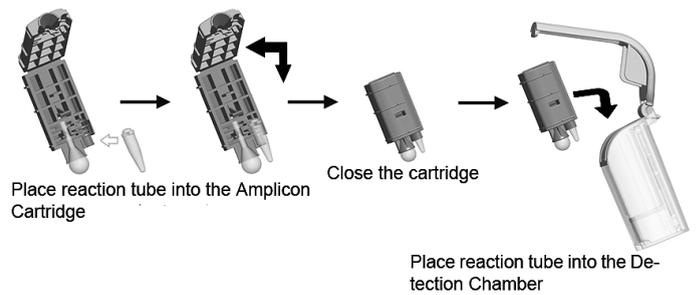
7. Place the reaction tube into the appropriate hole in the pre heated block (60°C) and incubate for exactly 1 hour. Do not open the tube cover during or at the end of the incubation period.

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 PCR^{un}® 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 PCR^{un} 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.

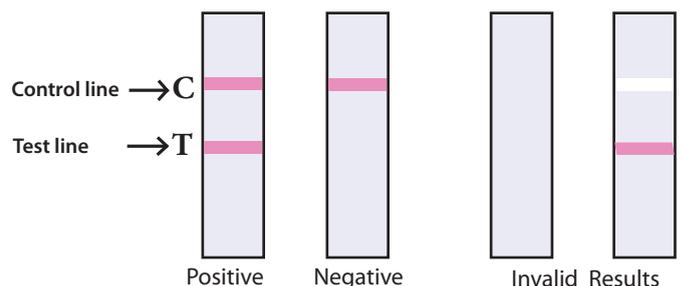


READING AND INTERPRETING THE RESULTS

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 *Anaplasma platys*.
2. **Negative Result** - a single control line appears. The appearance of a control line only, indicates the absence of the *Anaplasma platys* 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 treatment will most likely display a negative PCR result.

ANALYTICAL SENSITIVITY

The PCR^{un} reaction can detect 10³ copies of the target gene in pure DNA.

REFERENCES

1. Dumler, J.S.; Barbet, A.F.; Bekker, C.P.; Dasch, G.A.; Palmer, G.H.; Ray, S.C.; Rikihisa, Y.; Rurangirwa, F.R. (2001). Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of Ehrlichia equi and 'HGE agent' as subjective synonyms of Ehrlichia phagocytophilum. Int. J. Syst. Evol. Microbiol. 51 (6), 2145-2165.
2. Lima, M.L.F.I.; Soares, P.T.; Ramos, C.A.N.; Araújo, F.R.; Ramos, R.A.N.; Souza, I.I.F.; Faustino, M.A.G.; Alves, L.C.A. (2010). Molecular detection of Anaplasma platys in a naturally-infected cat in Brazil. Braz. J. Microbiol. vol.41 no.2 São Paulo
3. Renata Fernandes Ferreir; Aloysio de Mello Figueiredo Cerqueira; Ananda Müller Pereira; Cecília Matheus Guimarães; Alexandre Garcia de Sá, Fabricio da Silva Abreu; Carlos Luiz Massard; Nádia Regina Pereira Almosny (2007). Anaplasma platys Diagnosis in Dogs: Comparison Between Morphological and Molecular Tests. Intern J Appl Res Vet Med • Vol. 5, No. 3.



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