Feline Mycoplasma Molecular Detection Kit

Cat. No.30FMH116/30FMH148

For *in vitro* veterinarian diagnostic use only

User Manual

INTENDED USE

PCRun® Feline Mycoplasma Molecular Detection Kit is intended for detection of *Mycoplasma haemofelis* in **DNA** isolated from feline **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 16s rDNA gene. It is intended for the qualitative detection of *Mycoplasma haemofelis*. 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

The hemotropic mycoplasmas are Gram negative parasitic bacteria lacking cell walls and which have an affinity to the outer membranes of erythrocytes. Three species have been identified in cats: *M. haemofelis*, Candidatus *M. haemominutum* and Candidatus *M.turicensis*[1]. *Mycoplasma haemofelis* (formerly classified as *Haemobartonella felis*) is considered to be the causative agent of hemolytic Feline Infectious Anemia (FIA). The organism appears in blood smears as small (0.3–0.8 µm) coccoid bodies, sometimes forming short chains of 3 to 6 organisms. The hemolytic anemia caused by *M. haemofelis* is usually regenerative in nature unless this response is suppressed by an underlying disease such as Feline Leukemia Virus infection. Parasitemia is episodic and is directly coupled with decreased hematocrit levels at the time of increased parasitic load. Because of the cyclic parasitemia, organisms may be numerous, rare or undetectable in a given blood sample.

Transmission can occur through arthropod vectors such as lice, fleas, ticks, and mosquitoes as well as by transfer of infected blood (blood transfusions or use of contaminated needles or surgical instruments). Vertical infection and direct transmission associated with aggressive behavior between cats have been reported. Most cats infected with *M. haemofelis* become asymptomatic carriers and redevelop milder versions of the disease when under stress[2].

DIAGNOSIS

In the acutely sick feline, macrocytic and normochromic regenerative anemia are most common. Diagnostic symptoms include pale mucous membranes, splenomegaly, lethargy, anorexia, depression, weight loss and weakness. Hematocrit values in cats presenting with clinical signs of illness are often 50% of the normal. Fever occurs in some acutely infected cats and may be intermittent in chronically infected individuals. Evidence of coexisting disease may be present. A carrier phase can last for years in which the cats appear clinically normal and the organism is rarely detectable in the bloodstream. Early diagnosis and appropriate therapy are key to a good prognosis. Laboratory confirmation is traditionally accomplished by cytologic evaluation of the red blood cells. False negative results can occur as the number of infected cells fluctuates quickly and infection can easily be missed. An experienced eye is necessary to properly differentiate *Mycoplasma* organisms from artifacts in poorly stained slides; for this reason false positive results are common. Organisms detach from the erythrocytes in aged samples (approx. 24 hrs) and can be interpreted as stain precipitates leading to misdiagnosis. Polymerase Chain Reactions (PCR) such as PCRun have been developed with greater specificity and sensitivity than the subjective microscopic blood smear identification method. PCR reactions can detect pathogens in sample in which the organism is not present on the cell and are useful tools in identifying cats with low parasitemia[3].

KIT CONTENTS

Components	16 Test Kit	48 Test Kit
PCRun® strip of 8 lyophilized *Mycoplasma* single reaction tubes	2	6
PCRun® buffer to re-dissolve lyophilized reaction pellets	2 Vials, 200 µl	6 Vials, 200 µl
PCRun®lyophilized *Mycoplasma* Positive Control	1 vial	1 via
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 2-8^{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
- √ 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 -20^0 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 60^{0}C. 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 into the 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

1. Messick JB. Hemotrophic mycoplasmas (hemoplasmas): A review and new insights into pathogenic potential. Vet Clin Pathol. 2004;33:2-13.

2. Haemotropic mycoplasmas:What's their real significance in cats?. Journal of Feline Medicine and Surgery. 2010;12(5): 369-381.

3. Jensen WA, Lappin MR, Reagan W, et al. Use of a polymerase chain reaction assay to detect and differentiate two strains of Haemobartonella felis in
naturally infected cats. Am J Vet Res 2001;62:604-608.

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