Feline Mycoplasma Molecular Detection Kit

Cat. No.30FMH108

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 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. Seal with tape.**
- 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 regenerative 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	Contents	Amount
Aluminum pouch **Cat No. 03FMH100**	PCRun™ strip of 8 lyophilized *Mycoplasma* 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 µl*	10

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

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 optimal 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 PCRun™ strip from its protective pouch. Take care to return the unused tubes to the aluminium envelope and seal completely with tape 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 PCRun™ Sample Prep Kit into the 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 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 PCRUN™ 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 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.

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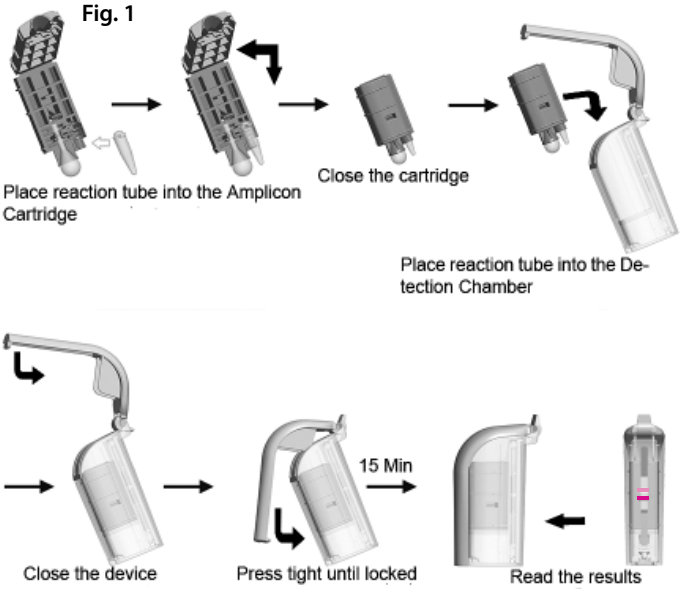
Fig. 1

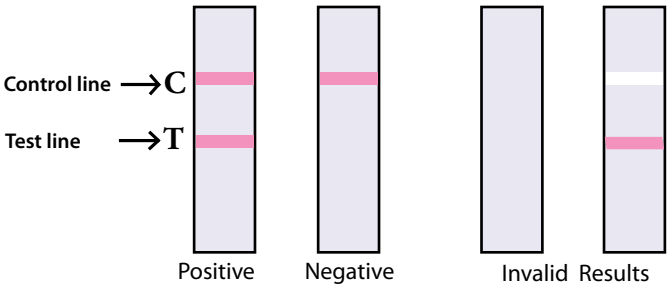


READING AND INTERPRETING THE RESULTS

A valid test must present a red control band. The control line must appear regardless of a positive or nehgative 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 *Mycoplasma haemofelis*.
2. **Negative Result** – a single control line appears. The appearance of a control line only, indicates the absence of the *Mycoplasma haemofelis* DNA or the that the copy number is below the detection limit.

Fig. 2



LIMITATIONS

As with any diagnostic test, results acquired with the PCRun™ Molecular Detection Kit should be interpreted in consideration of all clinical and laboratory findings. Animals undergoing antibiotic treatment will most likely display a negative PCR result.

ANALYTICAL SENSITIVITY

The PCRun™ reaction can detect 10^2 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.