Feline Mycoplasma Molecular Detection Kit
Cat. No.30FMH116/30FMH148
For in vitro veterinarian diagnostic use only
User Manual

INTENDED USE
PCR™ 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
PCR™ 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 PCR™ Reader.

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 PCR™ 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 PCR™ reaction tubes from the sealed pouches only immediately prior to their use.
- Return unused PCR™ 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. turicensis¹. 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) coccioid 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 stress².

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 PCR™ 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 parasitemia³.

KIT CONTENTS

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<thead>
<tr>
<th>Components</th>
<th>16 Test Kit</th>
<th>48 Test Kit</th>
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<tr>
<td>PCR™ strip of 8 lyophilized Mycoplasma single reaction tubes</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>PCR™ buffer to re-dissolve lyophilized reaction pellets</td>
<td>2 Vials, 200 μl</td>
<td>6 Vials, 200 μl</td>
</tr>
<tr>
<td>PCR™ lyophilized Mycoplasma Positive Control</td>
<td>1 vial</td>
<td>1 vial</td>
</tr>
<tr>
<td>Buffer to reconstitute and dilute positive control.</td>
<td>1 vial, 800 μl</td>
<td>1 vial, 800 μl</td>
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EQUIPMENT TO BE SUPPLIED BY USER:
- DNA extraction kit designed for use with PCR reactions
- PCR™ Reader aquired from Biogal
- Timer
- Small scissors
- Fine tipped permanent marker
- Protective laboratory gloves
- Accurate laboratory pipettes with aerosol barrier tips
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 10^3 copies of the target gene in pure DNA.

REFERENCES


Manufacturer: Biogal Galed Labs. Acs. Ltd.
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