

## 1. DESCRIPTION

*Mycoplasma haemocanis* (formerly *Haemobartonella canis*) is not a typical bacteria, but belongs to a group of microorganisms called mycoplasma, which are the smallest free-living type of 'germs'. *M. haemocanis* is a red blood cell parasite that causes disease mainly in immunosuppressed and splenectomized dogs. Clinical outbreaks of the disease occasionally occur.

In acute disease, the dog will usually show depression, loss of appetite, weight loss, and fever. In severe cases, death can occur. A chronic form of the disease has been reported rarely and may cause some weakness, an increase in appetite, and craving to eat unnatural articles such as rocks or dirt. Similar to *Mycoplasma haemofelis*, the bacteria can be transmitted from one animal to another by blood-feeding arthropods such as fleas and ticks. *M. haemocanis* infection is a widespread latent disease in kennel-raised dogs and infection rates can vary from approximately 30 % to 87 %. Although treatment of infected dogs with antibiotics may control acute infection, antibiotics cannot completely clear the bacteria in the blood, resulting in many infected dogs becoming carriers.

VetPCR™ MY.HAEMC Detection Kit is the direct detection of *Mycoplasma haemocanis* on the basis of a genetic database, so it can diagnose very fast and accurately. It can amplify only specific gene using the PCR (Polymerase Chain Reaction) method, and take only 3 hours for detection. Therefore, it is a very fast, accurate and reliable technique.

## 2. STORAGE

The components of VetPCR™ MY.HAEMC Detection Kit should be stored at -20°C. Under this condition, the kit is stable until expiration date stated on the label.

## 3. CONTENTS

	Kit 48	Kit 96	
VetPCR™ MY.HAEMC PCR Pre-mixture .....	48	96	tubes
DNase/RNase-free water .....	1	1	vial
MY.HAEMC PCR Positive control .....	1	1	vial
MY.HAEMC PCR Positive control Pre-mixture.....	4	8	tubes
Brig™ Molecular Weight marker .....	1	1	vial
Mineral Oil .....	1	1	vial
DNA purification kit (see step 6.1) .....	50	100	tests

## 4. SPECIMEN

0.5 ml whole blood in EDTA (purple top) tube.

## 5. ADDITIONAL REQUIRED MATERIALS

- Disposable gloves
- Pipettes
- Sterile pipette tip
- Vortex mixer
- Centrifuge for microcentrifuge tubes
- Thermal cycler
- Electrophoresis kit
- UV transilluminator

## 6. PROCEDURE

Please read through the entire procedure before starting.

### 6.1 DNA PREPARATION

Various manufacturers offer DNA isolation kits. Please carry out the DNA isolation according to the manufacturers instructions. The following standard DNA Purification kit is recommended.

Product	Catalog No.	Manufacturer
Bioingentech™ Genomic DNA Purification Kit (50 test)	230040(50)	Bioingentech Biotechnology Inc.
Bioingentech™ Genomic DNA Purification Kit (100 test)	230040(100)	Bioingentech Biotechnology Inc.

### 6.2 AMPLIFICATION

1.- Prepare appropriate PCR Premix tubes and one PCR Premix tube for Positive control. Label.

2.- Add 6µl of DNase/RNase-free water into the PCR Premix tube to total volume as 11µl.

3.- Add 2µl of template DNA into the PCR Premix tube to total volume as 13µl.

4.- Add 6µl of DNase/RNase-free water and 2µl of PCR Positive control into a PCR Positive control Premix tube for monitoring of amplification and easy interpretation.

5.- Add mineral oil (11µl). This step is necessary, even when using a thermal cycler that employs a top heating method.

6.- Perform PCR reaction of samples as the below process, using a PCR thermal cycler.

PCR cycle		Temp.	Time
1 Cycle	Initial Denaturation	94°C	2 min.
	Denaturation	94°C	30 sec.
	Annealing	55°C	30 sec.
30 Cycles	Extension	72°C	30 sec.
	Final extension	72°C	5 min.

### 6.3 DETECTION OF AMPLIFIED PRODUCTS

1.- Prepare 1.5% agarose gel containing Ethidium bromide (Et-Br).

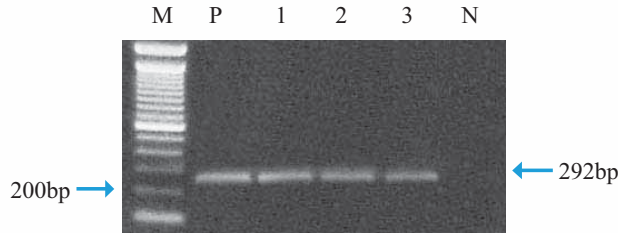
2.- Load 7µl of PCR product, 7µl of positive control and 2µl of Brig™ Molecular Weight marker on agarose gel without adding a loading-dye buffer and perform electrophoresis.

3.- Run electrophoresis by 100V (required about 30~40 minutes).

4.- Identify the result on ultra-violet (UV) transilluminator.

## 6.4 INTERPRETATION

- Expected PCR product size : 292 bp



**Fig1.** Electrophoresis of PCR product by VetPCR™ MY.HAEMC Detection Kit  
Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)  
Lane P : Positive control  
Lane 1~3 : MY.HAEMC Positive sample  
Lane N : Negative control

## 7. NOTICE

- For research purpose only. Not for use in diagnostic procedures for clinical purposes. *For in Vitro Use Only.*
- Take care in handling of specimen to minimize risk of infection.
- The PCR process is covered by patents issued and applicable in certain countries. Bioingentech Biotechnology Inc. does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

## 8. TROUBLE SHOOTING

- 1.- In the case of difficult to interpret results due to non-specific bands; reduce amount of template by 1/10 dilution, heated at 65° C for 5 min. and reacts again.
- 2.- Preparation of PCR reaction at room temperature may cause the non-specific band.
- 3.- All procedure should be carried out on ice.

## 9. ORDERING INFORMATION

Product	Catalog No.
VetPCR™ MY.HAEMC Detection Kit 48	VET0021C(48)
VetPCR™ MY.HAEMC Detection Kit 96	VET0021C(96)
Brig™ Molecular Weight Marker	24012



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