

Ehrlichia canis Detection test



Ehrlichia canis Detection Kit

Introduction

Ehrlichia, named for the Dr. Ehrlich who first described them, are a special type of bacteria which infect and live within the white blood cells of their hosts. Different types of Ehrlichia live in different types of white blood cells. Hosts can be human, pet, or wild animals. Ehrlichia are spread from host to host by tick bites and their intracellular location makes them difficult to remove as most antibiotics do not penetrate to the inside of cells.

Dogs and people are exposed to and susceptible to infection by many of the same tick-borne bacterial pathogens in the order Rickettsiales, including Anaplasma phagocytophilum, Ehrlichia canis, E. chaffeensis, E. ewingii, Rickettsia rickettsii, R. conorii, and other spotted fever group rickettsiae. Recent findings include descriptions of novel Ehrlichia and Rickettsia species, recognition of the occurrence and clinical significance of co-infection, and increasing awareness of Rhipicephalus sanguineus-associated diseases. Newer molecular assays are available, although renewed efforts to encourage their use are needed. This review highlights the ecology and epidemiology of these diseases, and proposes avenues for future investigation..

Principles

BIONOTE Ehrlichia canis Detection Test Kit is a complete, easy to use PCR kit for detection of the Fusion protein gene from Ehrlichia canis.

BIONOTE Ehrlichia canis Detection Test Kit is very accurate and highly efficient, and the PCR-reaction can be done from 50fg to 500ng of template DNA.

Materials provided (96 Reactions/Kit)

Cat. No. PD51-05

No.	Product	Amounts
1	E. canis Positive Template Control (Red cap)	200 $\mu\ell$ x 1 vial
2	E. canis Detection Solution(Brown cap)	500 $\mu\ell$ x 1 vial
3	Nuclease Free Water (Colorless cap)	1500 $\mu\ell$ x 1 vial
4	2 x PCR Master Mix (Blue cap)	1000 $\mu\ell$ x 1 vial

Precautions

- For veterinary research use only.
- For best results, follow the instruction sheet completely.
- All specimens should be handled as potentially infectious.
- Perform the PCR testing in an area separated from nucleic acid preparation or PCR product analysis. It is generally recommended that the reaction setup is performed on a clean bench.
- Do not open or remove test kits from their individually sealed pouches until immediately before use.
- Do not use the test kit if the pouch is damaged or the seal is broken.
- Do not use reagents beyond the stated expiration date marked on the package label.
- Use a precise micropipette with a sterilized filter tip.
- Perform the PCR testing at a clean bench to avoid contamination by any Nucleic acid or other PCR product.

Storage and Stability

- This kit is shipped at +2 to +15°C. Store the kit after arrival at -20°C or less in the dark.
- The test kit is stable through the expiration date marked on the package label.

Procedure of the test

1. Sample materials

Prepare the PCR tubes for 1 positive control, 1 negative control and a PCR tube for each of your samples (Template DNA).

NTC (Negative Template Control)

To detect a potential contamination, run a negative control every time the kit is used. Nuclease Free Water[®] should be used instead of template RNA.

PTC (Ehrlichia canis Positive control)

Ehrlichia canis Positive Template Control[®] is designed to react with the Ehrlichia canis primer set. This material consists of Ehrlichia canis DNA.

Test Specimen

Template DNA can be easily extracted by QIAamp DNA Mini Kit*. The procedures of Template DNA extraction should be referred to in the instructions of the chosen kit.

* QIAamp DNA Mini Kit: Manufactured by QIAGEN, USA

- Prepare a master mix by serially dispensing components to each tube in the following manner;

Reagents	Volume per reaction
Detection Solution ^②	N x 5 $\mu\ell$
2x Master Mix ^④	N x 10 $\mu\ell$
Total volume	15 $\mu\ell$

Set up reaction in PCR tubes by combining 15 $\mu\ell$ of the master mix and 5 $\mu\ell$ of negative control, positive control and samples.

- Perform the RT-PCR reaction under the below conditions.

Cycles	Reaction	Temp.(°C)	Time
1	Activation of Hot Start Taq DNA polymerase	95°C	12 min.
40	Denaturation	95°C	30 sec.
	Annealing	58°C	30 sec.
	Extension	72°C	40 sec.
1	Final extension	72°C	5 min.

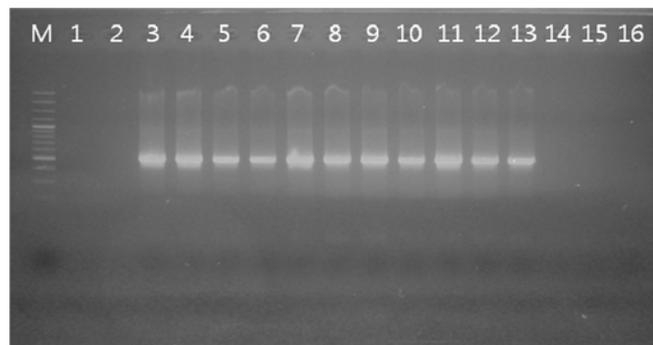
- Load PCR products on 1.5% agarose gel and perform the electrophoresis (at 100~120 Volts for 20 minutes).

PCR Product Size:

Target	Product size(bp)
16s rRNA	488 bp

Interpretation of the test

Compare the product size of the sample(s) with the size of the ladder and the positive control. If sample PCR product is detected at 266bp, it shows the sample is Ehrlichia canis positive.



Lane M: 100bp ladder size marker
 Lane 1: Negative control
 Lane 2: Extraction Blank
 Lane 3: Positive control
 Lane 4~13: Positive sample DNA
 Lane 14~16: Negative sample DNA

Limitations of the test

- This kit can detect all Ehrlichia canis. Although BIONOTE Ehrlichia canis Detection Test Kit is very accurate in detecting Ehrlichia canis DNA, a low incidence of false results can occur.
- As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the veterinarian after all clinical and laboratory findings have been evaluated.

Required materials and equipment not provided

- DNA Extraction Kit(QIAamp DNA Mini Kit)
- PCR tube(DNase Free)
- Disposable power free gloves
- Adjustable precise pipette(0.4~20 $\mu\ell$)
- Sterile filter pipette tips
- Thermal Cycler
- Gel electrophoresis system
- 1 x Electrophoresis buffer (TAE, TBE, TPE, etc.), Gel loading dye, Ethidium bromide, 1.5% Agarose gel, 100 bp DNA ladder
- UV trans-illuminator
- Ice or cooler unit

Date issued : Aug. 9, 2010

Doc. No.: I5105 -0E

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