

1. DESCRIPTION

Anaplasmosis is caused by a parasite called, *Anaplasma marginale*. It invades and multiplies in red blood cells. This parasite can be spread both mechanically and biologically. Mechanical transmission occurs by direct inoculation of cattle with blood-contaminated hypodermic needles, and surgical or dehorning instruments. Tabanids, including horseflies, may also mechanically transmit the disease on their mouthparts after taking a blood meal from an infected animal. Ticks are biological vectors of *A. marginale* and spread of the disease is most likely to occur through infected tick bites. The species of ticks important in spreading *A. marginale* are present in Alberta. Outbreaks of *Anaplasmosis* are usually seasonal and occur during or immediately after the vector season. As the disease progresses, infected red blood cells are destroyed causing anemia and occasionally death. Animals sick with *Anaplasmosis* may have a fever, and be off feed, depressed, dehydrated, show rapid or difficult breathing and have anemia. A few animals may also become excited or aggressive if enough oxygen doesn't reach the brain. Cattle of any age can become infected but calves rarely become sick. Infected calves may become carriers of the parasite. On the other hand, up to 30 to 50 percent of cattle that become infected at three years of age or older may die if they develop clinical signs of the disease.

VetPCR™ ANA.MARGI Detection Kit is the direct detection of *Anaplasma marginale* 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™ ANA.MARGI 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™ ANA.MARGI PCR Pre-mixture	48	96	tubes
DNase/RNase-free water	1	1	vial
ANA.MARGI PCR Positive control.....	1	1	vial
ANA.MARGI 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, or egg (albumen), thymus or spleen.

5. ADDITIONAL REQUIRED MATERIALS

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

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	55.5°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 : 517 bp

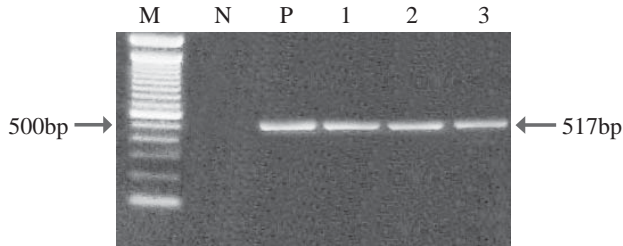


Fig1. Electrophoresis of PCR product by VetPCR™ ANA.MARGI Detection Kit
Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)
Lane N : Negative control
Lane P : Positive control
Lane 1~3 : ANA.MARGI Positive sample

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™ ANA.MARGI Detection Kit 48	VET0001B(48)
VetPCR™ ANA.MARGI Detection Kit 96	VET0001B(96)
Brig™ Molecular Weight Marker	24012



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