

1. DESCRIPTION

Neospora caninum is a recently discovered, apicomplexan, coccidial protozoan that associate with neonatal neurological and neuromuscular disease in mammals such as dogs, cattle, sheep and deer.

In infected neonatal dogs, progressive hind limb paresis and paralysis are the most common clinical signs. Skin involvement has only been reported in older dogs. The life cycle of this parasite consists of three stages known as tachyzoite, tissue cyst and oocyst. Tachyzoites are the rapidly multiplying form of the parasite that invades a variety of cells, producing the characteristic lesions of neosporosis in affected animals. The latent form is the tissue cyst, which contains bradyzoites and is found in peripheral and central nervous tissue.

Although other animals may be potential hosts of this parasite, only dogs can serve as both definitive (ie have tachyzoites in their tissues) and intermediate (ie shed oocysts in their feces) hosts of this parasite. When a definitive host ingests tissue cysts from infected intermediate host tissues, sexual development of this parasite takes place. This results in shedding of unsporulated oocysts in the feces. Sporulation occurs outside the host. Intermediate hosts such as cattle, dogs, sheep, goats, horses and deer may then become infected by ingesting food or water contaminated with the oocysts.

VetPCR™ NEO.CAN Detection Kit is the direct detection of *Neospora caninum* on the basis of a genetic data base, 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™ NEO.CAN 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 3 | Kit 5 | |
|--|-------|-------|-------|
| VetPCR™ NEO.CAN PCR Pre-mixture | 48 | 96 | tubes |
| DNase/RNase-free water | 1 | 1 | vial |
| NEO.CAN PCR Positive control | 1 | 1 | vial |
| NEO.CAN 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

1 ml feces, or 0.5 ml whole blood in EDTA (purple top) tube, or 0.5 ml aborted material, CSF, brain or heart tissue.

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.

5 . INTERPRETATION

- Expected PCR product size : 261 bp

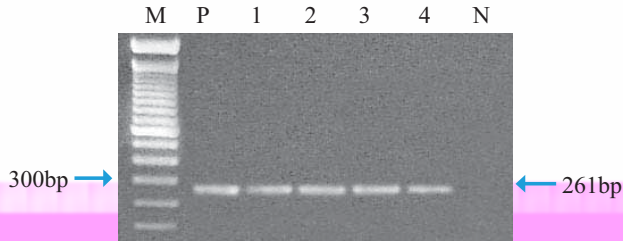


Fig1. Electrophoresis of PCR product by VetPCR™ NEO.CAN Detection Kit
 Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)
 Lane P : Positive control
 Lane 1~4 : NEO.CAN Positive sample
 Lane N : Negative control

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.

3. 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.

. ORDERING INFORMATION

| Product | Catalog No. |
|----------------------------------|--------------|
| VetPCR™ NEO.CAN Detection Kit 48 | VET0023C(48) |
| VetPCR™ NEO.CAN Detection Kit 96 | VET0023C(96) |
| Brig™ Molecular Weight Marker | 24012 |



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