

1. DESCRIPTION

Canine coronavirus (CCoV) is a cause of sporadic outbreaks of enteritis in dogs. The infection was first described by Binn in 1971 during an epizootic in Germany, although serological evidence was obtained earlier suggesting that dogs may be naturally infected with a coronavirus related to swine transmissible gastroenteritis virus (TGEV). CCoV is now recognized as an important, but imperfectly understood, pathogen of dogs. The incubation period is short. Vomiting and diarrhea may be seen by 1 - 3 days post-infection and, when clinical illness occurs, virus spreads rapidly. The virus is highly contagious and often may cause clinical signs in some dogs, with no illness occurring in other contact animals. Feces may be mucoid or watery, sometimes streaked with blood, and it is exceptionally malodorous. Pups become dehydrated, depressed and anorexic, even if fluid therapy is started early. There is generally no fever, although elevated body temperatures have been observed in some cases.

VetPCR™ CCoV Detection Kit is the direct detection of *Canine coronavirus* 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™ CCoV 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™ CCoV RT-PCR Pre-mixture..... | 48 | 96 | tubes |
| VetPCR™ CCoV PCR Pre-mixture..... | 48 | 96 | vial |
| BrigRT-PCR™ solution | 1 | 1 | vial |
| Biotech™ Transcriptase solution | 1 | 1 | vial |
| DNase/RNase-free water | 1 | 1 | vial |
| CCoV RT-PCR Positive control..... | 1 | 1 | vial |
| CCoV RT-PCR Positive control Pre-mixture..... | 4 | 8 | tubes |
| CCoV PCR Positive control Pre-mixture..... | 4 | 8 | tubes |
| Brig™ Molecular Weight marker | 1 | 1 | vial |
| Mineral Oil | 1 | 2 | vial(s) |
| RNA extraction kit (see step 6.1) | 50 | 100 | tests |

4. SPECIMEN

1 ml feces.

5. ADDITIONAL REQUIRED MATERIALS

- Pipettes, Sterile pipette tip, Vortex mixer
- Centrifuge for microcentrifuge tubes
- Thermal cyler, Electrophoresis kit, UV transilluminator

6. PROCEDURE

Please read through the entire procedure before starting.

6.1 RNA PREPARATION

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

| Product | Catalog No. | Manufacturer |
|--|-------------|---------------------------------|
| Bioingentech™ Total RNA Purification Kit (50 test) | 230041(50) | Bioingentech Biotechnology Inc. |
| Bioingentech™ Total RNA Purification Kit(100 test) | 230041(100) | Bioingentech Biotechnology Inc. |

6.2 AMPLIFICATION

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

2.- Add 5µl of DNase/RNase-free water into the RT-PCR Premix tube to total volume as 8,5µl.

3.- Add 1,5µl of template RNA into the RT-PCR Premix tube to total volume as 10µl.

4.- Add 5µl of DNase/RNase-free water and 1,5µl of RT-PCR Positive control into a RT-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 cyler that employs a top heating method.

6.- Perform RT-PCR reaction (RT-PCR 1) of samples as the below process using a PCR thermal cyler.

7.- Add 0,3µl of BrigRT-PCR™ solution and 0,5µl of Biotech™ Transcriptase solution.

8.- Perform RT-PCR reaction (RT-PCR 2) of samples as the below process, using a PCR thermal cyler.

| RT-PCR cycle | | Temp. | Time |
|--|--------|----------------------|--------------|
| RT-PCR 1 | 1Cycle | Initial Denaturation | 80°C 10 min. |
| | 1Cycle | Stop | 4°C 5 min. |
| Add 0,3µl of BrigRT-PCR™ and 0,5µl of Biotech™ Transcriptase | | | |
| RT-PCR 2 | 1Cycle | Denaturation | 80°C 10 min. |
| | 1Cycle | Annealing | 25°C 10 min. |
| | 1Cycle | Extension | 37°C 50 min. |

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

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

11.- Add 2µl of template (cDNA) into the PCR Premix tube to total volume as 13µl.

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

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

14.- Perform PCR reaction of samples as the below process, using a PCR thermal cyler.

| PCR cycle | | Temp. | Time |
|-----------|----------------------|--------|---------|
| 1 Cycle | Initial Denaturation | 94°C | 2 min. |
| 30 Cycles | Denaturation | 94°C | 30 sec. |
| | Annealing | 55,5°C | 30 sec. |
| | Extension | 72°C | 30 sec. |
| 1 Cycle | 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 7 μ l of PCR product, 7 μ l of Positive control and 2 μ l of BrigTM 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 : 622 bp

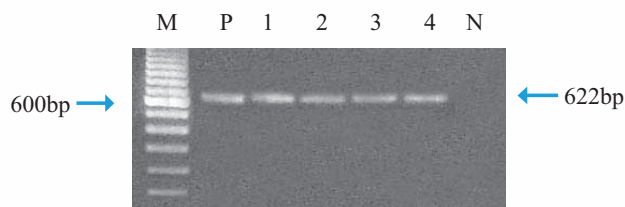


Fig 1. Electrophoresis of PCR product by VetPCRTM IPNV Detection Kit
 Lane M : BrigTM Molecular Weight Marker (Bioingentech Ltd.)
 Lane P : Positive control
 Lane 1~4 : IPNV Positive sample
 Lane N : Negative control

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 TM CCoV Detection Kit 48 | VET0001CR(48) |
| VetPCR TM CCoV Detection Kit 96 | VET0001CR(96) |
| Brig TM Molecular Weight Marker | 24012 |



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