

1. DESCRIPTION

Two distinct parvoviruses (CPV), are now known to infect dogs - the pathogenic CPV-2, which was recognized as a new disease of dogs and wild canines in 1978, and the “canine minute virus” (CMV, CPV-1) reported by Binn in 1970. MVC, a completely different parvovirus, had not been associated with natural disease until 1992. CMV may cause pneumonia, myocarditis and enteritis in young pups, or transplacental infections in pregnant dams, with embryo resorptions and fetal death. Confirmed infections have been reported in the USA, Sweden, Germany, and, more recently in Italy. Only about 30 cases have been reported. Canine parvovirus (CPV, CPV-2) and feline panleukopenia virus (FPV) are very closely related and are important pathogens of their respective hosts, the dog and cat. CPV-2 infects dogs and other *Canidae* such as wolves, coyotes, South American dogs and Asiatic raccoon dogs, but not cats. FPV and the FPV-like viruses infect both large and small cats, as well as mink, raccoons, and possibly foxes, but not dogs. However, the clear separation of a cat virus infecting only cats (FPV) and a dog virus infecting only dogs (CPV-2) is no longer certain as the original dog virus, CPV-2 was transitory, and replaced in nature by so-called “new antigenic types” (CPV-2a and CPV-2b) that infect or replicate in, and are transmitted between, dogs and cats.

Clinical signs of CPV are well known, and only briefly reviewed here since they have been reviewed in several publications. Disease is often asymptomatic in older dogs or in pups that receive a low virus dose since the severity of infection is highly dose related. For example, a pup may acquire infection by CPV in a contaminated kennel, dog show, or veterinary clinic and experience only mild, or no illness. However, virus amplified in the intestine of that pup would be shed in large amounts to littermates or other susceptible dogs in contact. In contrast to the marked panleukopenia seen in cats infected with FPV, a relative lymphopenia, not panleukopenia, is often observed in dogs infected with CPV. Lymphocyte numbers decline, but there is little effect on eosinophil, basophil, monocyte, or red cell numbers.

VetPCR™ CPV Detection Kit is the direct detection of *Canine parvovirus type-1/type-2* 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™ CPV 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™ CPV PCR Pre-mixture	48	96	tubes
DNase/RNase-free water	1	1	vial
CPV PCR Positive control	1	1	vial
CPV 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 1 ml feces or 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	56°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 : 435 bp

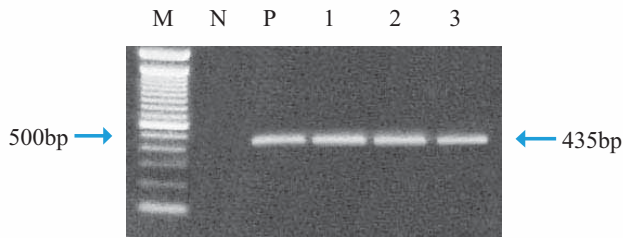


Fig1. Electrophoresis of PCR product by VetPCR™ CPV Detection Kit
 Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)
 Lane N : Negative control
 Lane P : Positive control
 Lane 1~3 : CPV Positive sample

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™ CPV Detection Kit 48	VET0014C(48)
VetPCR™ CPV Detection Kit 96	VET0014C(96)
Brig™ Molecular Weight Marker	24012



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