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# Comparative study for efficacy of Immunochromatography antigen assay (ICA) test kit with Polymerase chain reaction (PCR) to detect canine parvovirus

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**Abstract**
Canine parvovirus infection is an acute highly contagious life-threatening disease. It affects young puppies between the times of weaning to six months of age and therefore, diagnosis is essential for screening infected puppies at early stage to prevent mortality. The objective of the present study was to compare sensitivity and specificity of immunochromatographic antigen assay (ICA) test with gold standard polymerase chain reaction. Fecal samples of one hundred dogs suspected for canine parvovirus were tested with Ubio quick VET rapid antigen test kit of which twenty two dogs (22) and in polymerase chain reaction thirty (30) dogs were found positive. Eight dogs which were detected negative with the ICA kit test were found positive for canine parvovirus with polymerase chain reaction. Relative to PCR results, sensitivity and specificity of ICA test kit was 73.33 percent and 100 percent respectively.

**Keywords:** Canine parvovirus, ICA, PCR, sensitivity, specificity

## Introduction
Parvovirus belongs to the family *parvoviridae,* subfamily *parvovirinae* and genus parvovirus. It is non-enveloped, icosahedral, smallest DNA virus with the virion exhibiting a diameter between 18 to 26 nm comprised of linear, single standard DNA of about 5.2 kb encoding two structural (VP1 and VP2) and two non-structural (NS1 and NS2) proteins (Ford *et al.,* 2017, Mira *et al*., 2017) [7, 11]. This highly contagious, often fatal disease is caused by strains of CPV-2 (2a, 2b and 2c) (Garcia *et al*., 2016) [8]. The canine parvovirus infected dogs exhibits clinical signs such as lethargy, dehydration, anorexia, fever, vomiting and diarrhea which may contain mucus or blood with a very strong foul, smell (Ogbu *et al.,* 2017) [12]. Feces constitutes as the most suitable material in the enteric form of the disease because it may contain >10 $^{9}$/gm virus particles that can be shed during the acute phase (Carmichael and Binn, 1981) [4]. For diagnosis of canine parvovirus (CPV) immunochromatographic antigen assay (ICA) based test kits has been used in fecal samples of dogs, since it is a rapid, reproducible, sensitive and easy to perform even by the dog owners with minimal costs (Esfandiari and Klingeborn, 2000, Vakili *et al*., 2014) [6, 19]. Recently the polymerase chain reaction (PCR) technique has been increasingly used as a tool for the diagnosis of CPV infection. It is a reliable technique with high degree of sensitivity and specificity in detecting CPV from fecal samples than the antigen or antibody based methods (Kumar and Nandi, 2010, Sriniwas *et al*., 2013) [10, 17]. The polymerase chain reaction could detect 10 $f_g$ of viral replicative form (RF) DNA on agarose gel electrophoresis (Schunck *et al*., 1995) [16]. PCR is useful when fecal antigen tests are negative but parvovirus enteritis is still suspected according to clinical symptoms as a diagnosis. However, PCR needs relatively expensive equipment and reagents, which are not available in routine veterinary practice. This has led to the development of various rapid field level diagnostic test kits based on the principle of ICA test (Vakili *et al*., 2014) [19].

## Material and Method
### Sample collection
A total of hundred dogs, (different gender, age, breed) showing signs of diarrhea (haemorrhagic or non- haemorrhagic) and vomiting which were presented in TVCC, College of Veterinary and Animal Science, Bikaner were selected for the present study. Fecal samples were collected with sterile rectal swabs for the detection of parvoviral antigen by ICA kit and

faecal sample was also stored in phosphate buffer saline (pH=7.2) at -20 $^{0}$C for further confirmation by polymerase chain reaction method.

### Immunochromatographic assay (ICA) method

Canine parvovirus antigen in the fecal sample was detected using CPV antigen rapid test kit following the manufacturer's instructions. In this method, fecal sample was collected directly from rectum and mixed with assay diluent provided with the test kit. Thus prepared diluent was taken in a pipette and 4-5 drops of the same was added on sample area of the test card. The test was read after 5 minutes. If there are enough parvoviral antigens in the sample, a visible T band appeared and gave positive result.

### Polymerase chain reaction method

### Sample preparation and DNA isolation

DNA was manually isolated by Phenol-Chloroform Isoamyl (PCI) method by Barker (1998) [1]. The fecal suspensions with PBS had been processed by centrifuging at 1500 rpm for 10 min at 4°C. Then supernatants have been collected in 2 ml series tube. The supernatant (500 µl) was boiled for 10 min at 100°C to inactivate the polymerase chain reaction (PCR) inhibitors and chilled on ice. After boiling the sample was centrifuged at 1500 rpm for 5 min and the collected supernatant was used as a source of DNA template for PCR. The lysate was stored at −20°C until further use.

### Amplification of CPV (VP2 gene) in feces by PCR

The DNA extracts also were tested by a VP2 gene of CPV PCR assay, following the method developed by Pereira *et al.* (2000). VP2 partial gene of CPV amplified by conventional PCR by the using already published primer 555-Forward-AGGAAGATATCCAGAAGGA and 555-Reverse-GGTGCTAGTTGATATGTAATAAACA (Buonavoglia *et al.,* 2001) (Table-1). The primers amplified portion of VP2 gene of CPV to yield a product size of 583bp. The reaction mixture for PCR was prepared in 200µl thin walled PCR tubes (Table-3). The master mix was prepared for each sample by adding 20 µl of Nuclease Free Water (NFW), 25 µl PCR master mixture, 1 µl of forward and 1 µl reverse primers each and the template DNA (3 µl) was separately added in the tubes. The 50 µl reaction mixture was kept for amplification in programmed thermal cycler (Table- 2). The PCR amplification conditions were as follows: initial denaturing at 95°C for 5 min followed by 36 cycles of 95°C for 30 s, annealing 55°C for 30 s, and extending at 72°C for 30 s, with a final extension at 72°C for 10 min. After the amplification, the PCR product was stored at -20˚c for further analysis. The amplified PCR products were analyzed on 1% agarose gel visualized under UV transilluminator as per the procedure described by Sambrook and Russel (2001) [15].

**Table 1:** Primer sequence and position of Oligonucleotide used for the amplification of VP2 gene of CPV

| Primer | Primer Sequence | Sense | Position | Amplicon Size |
|---|---|---|---|---|
| 555-for$^b$ | CAGGAAGATATCCAGAAGGA (20mer) | + | 4003-4022 | 583bp |
| 555-rev$^b$ | GGTGCTAGTTGATATGTAATAAACA (25mer) | _ | 4561-4585 | |

**Table 2:** PCR reaction condition

| PCR Steps | Temperature | Time |
|---|---|---|
| Initial denaturation | 95°C | 5 min |
| Final denaturation | 95°C | 30 seconds |
| Annealing | 55°C | 30 seconds |
| Initial extension | 72°C | 30 seconds |
| Final extension | 72°C | 10 min |

**Table 3:** PCR components for each reaction

| S. No. | PCR Components | Quantity |
|---|---|---|
| 1. | 2x PCR assay buffer MgCl2 (4mM), dNTP (0.4mM),Taq DNA polymerase(0.05UµL) | 25 µl |
| 2. | Primer-F | 1 µl |
| 3. | Primer-R | 1 µl |
| 4. | Template DNA | 3 µl |
| 5. | Nuclease free water | 20 µl |
| 6. | Total | 50 µl |

### Statistical analysis

To compare the sensitivity, specificity and overall agreement between the viral detection assays viz. immunochromatographic assay test kit and Polymerase chain reaction, the statistical formula given by Samad *et al.* (1994) [14] was used (table 4).

**Table 4:** The statistical formula

| The test to be compared | Gold Standard test | | |
|---|---|---|---|
| | | Positive | Negative |
| | Positive | a | b |
| | Negative | c | d |
| | Total | a + c | b + d |

The notations in the table are defined as under:

a = Number of samples positive to both conventional and the gold standard test

b = Number of samples positive to conventional but negative to the gold standard test

c = Number of samples negative to conventional but positive to the gold standard test

d = Number of samples negative to both conventional and the gold standard test

### Result and Discussion

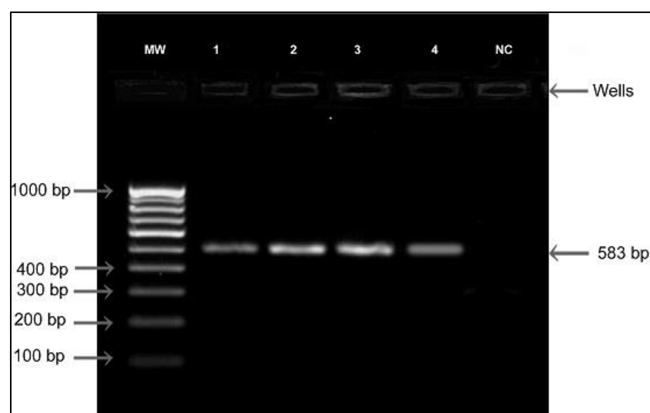
Fecal samples of one hundred dogs suspected for CPV were tested with CPV rapid antigen (ICA) test kit of which twenty two dogs (22) and in polymerase chain reaction thirty (30) dogs were found positive. Eight dogs which were detected negative with the ICA kit test were found positive for CPV with PCR. Relative to PCR results, sensitivity and specificity of ICA test kit was 73.33 percent and 100 percent respectively. Overall agreement between the two assays was 92 per cent (Table 5). Similar studies have been carried out by Tinky *et al.*, 2015 [18]; Hasan *et al.*, 2016 [9] and Bhargavi *et al.*, 2017 [2] and these workers stated that polymerase chain reaction is more sensitive, specific rather than ICA test kit. They found 44%, 46.1% and 79.61% positive from PCR and 36%, 20% and 58.33% positive from ICA test kit. The low sensitivity of the ICA test was associated with the low amount of virus shed in the feces during the later stages of disease and the presence of high CPV antibody titers in the gut lumen that may sequestrate most viral particles. Molecular methods like polymerase chain reaction (PCR) were developed that displayed high increased sensitivity and specificity (Desario *et al.*, 2005) [5]. Considering the sensitivity limits of the ICA

tests that have been observed previously, negative results from the test kit should be confirmed by PCR-based methods. The ICA provides a reliable method for screening where laboratory support and personnel are limited. However, large amounts of viral antigen are required to produce a clearly visible band and the interpretation of results may be affected by the subjectivity of a test operator.

**Table 4:** Sensitivity and specificity of ICA test kit in comparison to polymerase chain reaction

| Test | Polymerase chain reaction | | | Sensitivity | Specificity | Overall agreement |
|---|---|---|---|---|---|---|
| | | Positive | Negative | | | |
| ICA test kit | Positive | 22 | 0 | 73.33% | 100% | 92% |
| | Negative | 8 | 70 | | | |

**Fig 1:** Canine parvovirus ICA positive test kit




**Legends:** MW: Molecular weight marker,
**Wells:** Amplicons (583 bp), NC: Negative control

**Fig 2:** Polymerase chain reaction amplification product (VP2 gene)

## Conclusion

A rapid diagnosis of CPV-2 infection is important in kennels and shelters in order to isolate infected dogs and prevent secondary infections of susceptible contact animals. Since a clinical diagnosis is not definitive, several laboratory methods have been developed to detect CPV-2 in the feces of infected dogs. In this present study relative to polymerase chain reaction results sensitivity and specificity of ICA test kit was 73.33 percent and 100 percent respectively. Polymerase chain reaction having high specificity and sensitivity than the antigen or antibody-based methods. However, PCR requires expensive equipment, reagents and specialized laboratory which restricts its use in field level. The ICA test kits which are sensitive, simple and rapid are easy to perform without any specialized equipment, any specialized laboratory in a field condition by pet owners and veterinarians. In a conclusion of this study that for rapid screening of CPV in dogs in field condition ICA is very useful but polymerase chain reaction is more reliable and accurate then immunochromatographic assay.

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