SHORT COMMUNICATION

Comparative Evaluation of Two DNA Extraction Methods for Molecular Detection of Canine Parvo Virus -2

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Abstract

The molecular detection of Canine Parvo Virus-2 (CPV-2) - a highly contagious and potentially fatal virus in dogs- relies heavily on the quality and integrity of extracted viral DNA. In this study, we compared and evaluated the efficacy of two DNA extraction methods - Kit-based (HipurA® DNA stool purification kit) and Phenol-Chloroform-Isoamyl Alcohol (PCI) on 200 clinical faecal samples collected for CPV-2 detection. All 200 faecal samples were examined through PCR using CPV-2 primer sets. The examination revealed 147 (73.5%) samples by Kit and 153 (76.5%) samples by PCI positive for CPV-2 at 379 bp. The DNA extracted by both methods was run for agarose gel electrophoresis. The DNA concentration, purity (A260/280 and A260/230, A280) and overall yield were assessed using a Nanodrop spectrophotometer. Statistical analysis revealed significant differences between the methods. The PCI method yielded significantly higher DNA concentration (300.11 ± 87.75 ng/µL) than the Kit method (72.29 ± 25.28 ng/µL; p = 0.016). Protein purity (A260/280 ratio) was also superior in the PCI method (1.644 ± 0.058) compared to the KIT method (1.374 ± 0.058; p = 0.0028). However, both methods showed similar salt and organic contamination levels (A260/230), with no significant difference (p = 0.7418).

Key words: Canine parvovirus-2, DNA extraction, KIT method, Phenol-chloroform-isoamyl alcohol, Molecular diagnostics.

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Introduction

The accurate detection of viral pathogens is fundamental to both clinical diagnostic and virological research. In particular, the molecular detection of Canine Parvo Virus-2 (CPV-2) - a highly contagious and potentially fatal virus in dogs - relies heavily on the quality and integrity of extracted viral DNA. Among the various extraction methods available, kit based protocol and conventional Phenol-chloroform-isoamyl alcohol (PCI) method are commonly employed. The kit based extraction method is widely favoured for its standardised and streamlined procedures that enhances reproducibility and minimize the risks associated with handling hazardous reagents. Commercial extraction kits incorporate proprietary lysis buffer, binding columns and elusion reagent, which collectively facilitate rapid and efficient extraction of nucleic acids. Such operational simplicity enables high-throughput processing; making kit-based methods ideally suited for clinical environments where time and consistency are crucial (Boom *et al.,* 1999; Sloan *et al.,* 2021; Galla *et al.,* 2024). The commercial kits are faster and reliable then other methods but the yield of DNA varied and depends on individual kit (Desai *et al.,* 2020; Khan *et al.,* 2023).

In contrast, the phenol chloroform isoamyl (PCI) method remains a staple in molecular biology laboratories due to its ability to yield high-quality DNA with minimum contamination from inhibitors when performed meticulously. Despite the labour-intensive nature and the handling of toxic chemicals the PCI method allows for comprehensive cell lysis and effective of nucleic acid from protein and other cell components (Sambrook and Russell, 2001). However, its manual execution can lead to variability across experiments, which may affect downstream applications. The success of polymerase chain reaction (PCR)-based detection relies heavily on the quality and purity of the extracted viral DNA. Therefore, the choice of DNA extraction method is a critical factor influencing diagnostic accuracy (Wang *et al.,* 2019; Ghosh *et al.,* 2023; Gand *et al.,* 2023; Kurnosov *et al.,* 2025). This study presents a comparative evaluation of these two methods, with an emphasis on their methodological intricacies and performance in CPV-2 DNA extraction.

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Materials and Methods

Sample Collection

A total of 200 faecal samples swabs were collected from the dogs that were brought for treatment at Veterinary Clinical Complex, Post Graduate Institute of Veterinary Education and Research, Jamdoli, Jaipur (India) from different places in and around Jaipur. The dogs having clinical history of lethargy, dehydration, anorexia, fever, with progression to vomiting, diarrhoea and haemorrhagic diarrhoea were suspected for CPV-2. All the samples were collected directly from rectum of diarrheic dogs via sterile cotton swabs (Himedia®) which were preserved in sterile tubes containing 1% phosphate buffer saline (PBS) solution and transferred to -20°C deep freezer until further processed.

DNA Extraction

DNA extraction was conducted by two methods, one by using the kit and protocol described by Nandi and Kumar (2010), and another by HiPurA DNA Stool Purification Kit (Himedia), **t**he manufacturer's protocol was strictly followed. The extracted DNA was stored at -20°C for subsequent molecular detection assays.

Polymerase Chain Reaction (PCR)

PCR amplification was carried out using a CPV-2a–specific primer set described by Kaur *et al*. (2014), yielding a 379 bp amplicon. The forward primer (F) sequence was 5'-AGAGCATTGGGCTTACCACC-3' and the reverse primer (R) sequence was 5'-ATCTTCCTGTATCTTGATGTGCT-3'. The PCR reaction was performed in a final volume of 10 µL, consisting of 5 µL of 2× Taq PCR Master Mix (Qiagen), 0.5 µL each of forward and reverse primers, 2 µL of template DNA, and 2 µL of nuclease-free water. The thermal cycling conditions included an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min, with a final extension step at 72 °C for 10 min.

Agarose Gel Electrophoresis

PCR products were analysed by 1.2 % agarose gel electrophoresis, stained with 6 µL of ethidium bromide. The Nobivac Puppy DP® vaccine containing the CPV-2 strain (MSD Animal Health Pvt. Ltd.) was used as the positive control. The 50 positive samples DNA (n=50) from the both methods were run on the agarose gel via agarose gel electrophoresis, visualized using a gel Documentation system (Vilber fusion solo S) and images were captured using a thermal scanner. DNA concentration of 50 positive samples and purity were estimated using Nanodrop spectrophotometer.

Results and Discussion

All 200 faecal samples were examined through PCR using CPV-2 primer sets. The examination revealed 147 (73.5%) samples by Kit and 153 (76.5%) samples by PCI positive for CPV-2 at 379 bp (Fig. 1). The DNA bands obtained from the kit appeared sharp and well-defined (Fig. 2). Similarly, the bands from the PCI method showed comparable intensity but with slight variations (Fig. 3), suggesting minor differences in DNA concentration or integrity. The Statistical analysis of DNA concentration, purity (A260/280 and A260/230), and overall yield assessed using a Nanodrop spectrophotometer revealed significant differences between the methods. The PCI method yielded significantly higher DNA concentration (300.11 ± 87.75 ng/µL) than the Kit method (72.29 ± 25.28 ng/µL, $p = 0.016$). Protein purity (A260/280 ratio) was also superior in the PCI method (1.644 ± 0.058) compared to the Kit method (1.374 ± 0.058), showing high statistical significance ($p = 0.0028$). However, both methods showed similar salt and organic contamination levels (A260/230), with no significant difference ($p = 0.7418$). The PCI method's higher absorbance at 280 nm (A280) further confirmed enhanced purity, with significant variation from the Kit method ($p = 0.0254$)(Table 1). This comparison highlights the PCI method's superiority in DNA concentration and protein purity for CPV-2 detection, recommending its use in molecular diagnostics to enhance assay sensitivity and reliability.

The present study demonstrated that both DNA extraction methods were effective for CPV-2 detection; however, notable differences in DNA quality and yield were observed. The higher positivity rate and significantly greater DNA concentration obtained with the PCI method indicate improved recovery of viral nucleic acids from faecal samples. Superior A260/280 ratios further suggest reduced protein contamination, contributing to better PCR performance and consistency. Although the kit-based method produced sharp bands and offered procedural convenience, its lower yield may limit sensitivity in low viral load samples. Overall, the PCI method provides enhanced diagnostic reliability for CPV-2 molecular detection.

Table 1: Mean (±SE) values and statistical analysis of DNA extraction methods (n=50)

Parameter	KIT method	PCI method	t-value	P value
Concentration	72.28 ± 25.27	300.117 ±87.75	-2.57	0.0160*
A260/280	1.37 ± 0.058	1.644 ± 0.058	-3.28	0.0028*
A260/230	0.95 ± 0.109	1.012 ± 0.146	-0.33	0.7418
A280	1.01 ± 0.308	3.382 ± 0.984	-2.36	0.0254*

*Significant, p value <0.05

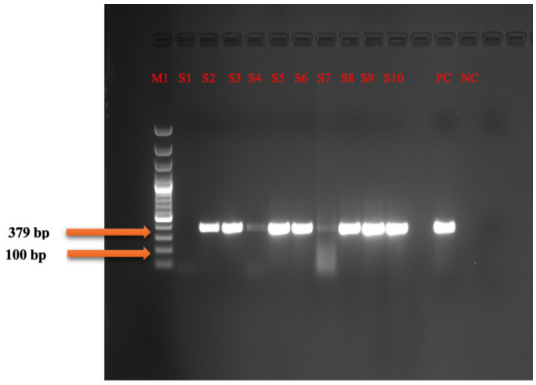

Fig. 1: PCR detection of CPV-2: Gel electrophoresis image showing CPV-2-positive samples obtained using both Kit and PCI DNA extraction methods. The expected amplicon size is 379 bp. S1 to S5 Kit samples, S6 to S10 PCR samples, M1 marker, PC Positive control (Puppy DP), NC Negative control (New)

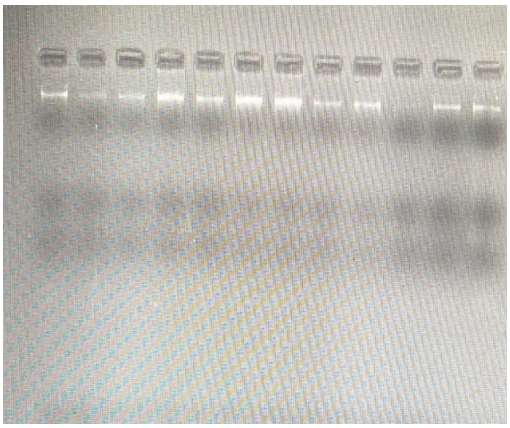

Fig. 2: DNA bands from Kit Method (Agarose gel electrophoresis results for DNA extracted using the HiPurA DNA stool purification kit. The bands represent DNA yield and purity levels obtained from clinical samples)

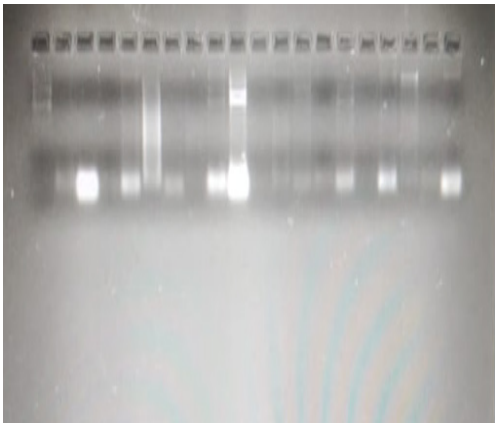

Fig. 3: DNA bands from PCI Method (Agarose gel electrophoresis results for DNA extracted using the PCI method. The bands indicate a higher yield and purity compared to the Kit method)

In conclusion, PCI Method yields significantly higher DNA concentration, and better protein purity (A260/280 ratio), in detecting CPV-2 from faecal samples using PCR. Although the kit method offers ease and speed, the PCI method is more suitable for research and diagnostic applications where high-quality DNA is essential for accurate CPV-2 detection.

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