MEDS Clinical Medicine (2025)
Clausius Scientific Press, Canada

DOI: 10.23977/medsc.2025.060504
ISSN 2616-1907 Vol. 6 Num. 5

Diagnostic Efficacy of a Combined Antigen Rapid Test for CPV, CCV, and Giardia in Canine Feces and Vomit Specimens

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Keywords: Canine Parvovirus (CPV); Canine Coronavirus (CCV); Giardia Lamblia; Antigen Combo Rapid Test; Feces; Vomit; Chromatographic Immunoassay; Veterinary Diagnostics

Abstract: The CPV+CCV+Giardia Antigen Combo Rapid Test Cassette is a chromatographic immunoassay designed for the qualitative simultaneous detection of canine parvovirus (CPV) antigen, canine coronavirus (CCV) antigen and Giardia lamblia antigen in feces and vomit specimens from dogs. This study aimed to do the in-house clinical studies of CPV+CCV+Giardia Antigen Rapid Test Cassette (Feces/Vomit) with the CPV+CCV+Giardia positive specimen and CPV+CCV+Giardia negative specimen. A total of 423 specimens (209 feces and 214 vomit) were collected, including clinically confirmed positive and negative specimens for CPV, CCV and Giardia. The results demonstrated high relative sensitivity (CPV: 95.74%, CCV: 93.10%, Giardia: 95.12%), relative specificity (CPV: 100.00%, CCV: 97.52%, Giardia: 95.87%) and accuracy (CPV: 97.56%, CCV: 96.09%, Giardia: 95.68%) across all three targets, with narrow 95% confidence intervals (CIs) indicating robust reliability. The test exhibited consistent performance in both feces and vomit specimens, with results readable within 5-10 minutes, making it suitable for point-of-care settings. These findings indicate that the CPV+CCV+Giardia Antigen Combo Rapid Test Cassette is a reliable, rapid and practical tool for the simultaneous detection of multiple enteric pathogens in dogs, aiding in timely diagnosis and targeted treatment.

1. Introduction

Enteric infections in dogs caused by canine parvovirus (CPV), canine coronavirus (CCV) and Giardia lamblia are major contributors to morbidity and mortality, particularly in puppies and immunocompromised animals[1]. CPV, a member of the Parvoviridae family, causes severe hemorrhagic enteritis and myocarditis, with mortality rates reaching 91% in untreated cases [2]. CCV, an enveloped RNA virus of the Coronaviridae family, induces mild to severe diarrhea, often exacerbating disease severity when co-infecting with CPV [3]. Giardia lamblia, a flagellated protozoan, is a common cause of chronic diarrhea in dogs, characterized by malabsorption and

weight loss, with zoonotic potential posing risks to human health [4].

The clinical manifestations of these infections-including diarrhea, vomiting, lethargy and anorexia-often overlap significantly, making differential diagnosis based solely on clinical signs challenging [5]. Accurate and rapid identification of the causative agent is critical for several reasons: CPV requires intensive supportive care (e.g., fluid therapy, antiemetics), CCV may benefit from antiviral interventions and Giardia responds to specific antiparasitic drugs (e.g., fenbendazole)[6]. Delayed or incorrect diagnosis can lead to disease progression, secondary infections and increased treatment costs [7].

Traditional diagnostic methods for these pathogens have limitations. Virus isolation for CPV and CCV is time-consuming (2-7 days) and requires specialized laboratory facilities, while microscopic examination for Giardia cysts is labor-intensive and has low sensitivity (50-70%)[8]. Polymerase chain reaction (PCR) is highly sensitive and specific but involves complex procedures, trained personnel and longer turnaround times (4-24 hours), limiting its utility in point-of-care settings such as small animal clinics or field conditions [9].

Chromatographic immunoassays have emerged as a valuable alternative for rapid antigen detection, offering advantages such as simplicity, portability and quick results (within 10-15 minutes) [10]. Single-target antigen tests for CPV, CCV or Giardia are widely available, but they require multiple tests to rule out co-infections, increasing cost and time. The CPV+CCV+Giardia Antigen Combo Rapid Test Cassette addresses this gap by enabling simultaneous detection of all three pathogens in a single assay. However, comprehensive evaluation of its performance is essential before widespread clinical application.

This study aimed to assess the diagnostic performance of the CPV+CCV+Giardia Antigen Combo Rapid Test Cassette using feces and vomit specimens, with commercial Anigen Rapid Test Kits as reference methods. We evaluated relative sensitivity, relative specificity and accuracy and discussed its clinical utility in veterinary practice.

2. Materials and Methods

2.1 Specimen Collection

A total of 423 specimens were collected from dogs with suspected enteric infections, including 209 feces and 214 vomit samples. The distribution of specimens for different pathogens was as follows: for CPV, there were 23 positive feces, 24 positive vomit, 17 negative feces and 18 negative vomit; for CCV, 29 positive feces, 29 positive vomit, 60 negative feces and 61 negative vomit; and for Giardia, 20 positive feces, 21 positive vomit, 60 negative feces and 61 negative vomit.

All specimens were clinically confirmed using a combination of PCR (for CPV and CCV) and microscopic examination with immunofluorescence (for Giardia) as gold standards, where positive specimens were defined as those with confirmed presence of the target pathogen by gold standard methods and negative specimens as those with no detectable pathogen. Specimens were collected using sterile containers, labeled with unique identifiers (e.g., CPV001, CCV023, Giardia015) and transported to the laboratory within 4 hours of collection. If not tested immediately, specimens were stored at 2-8 ℃ for up to 24 hours; long-term storage (-20 ℃) was avoided to prevent antigen degradation.

2.2 Test Kit and Procedure

The CPV+CCV+Giardia Antigen Combo Rapid Test Cassettes used in this study were manufactured by Hangzhou AllTest Biotech Co., Ltd., each kit contains test cassettes, extraction buffer, disposable droppers and detailed instructions for use. The test is based on the double

antibody sandwich technique: colloidal gold-conjugated monoclonal antibodies specific to CPV, CCV and Giardia antigens act as detectors, while corresponding specific antibodies are pre-coated on three separate test lines (T for CPV, C for CCV, G for Giardia) on the cassette. A control line (C) is also pre-coated to verify the validity of the test procedure.

The test procedure was performed strictly according to the manufacturer's package insert: firstly, the test kits, clinical specimens (feces or vomit) and extraction buffer were equilibrated to room temperature (15-30℃) for 30 minutes; secondly, for specimen preparation, 100 μl of feces or vomit was mixed with 1 ml of extraction buffer in a sterile tube, vortexed thoroughly for 30 seconds and allowed to stand for 2 minutes to form a homogeneous suspension; then the test cassette was placed on a clean and level surface, and using a disposable dropper held vertically, lastly, 3 drops (approximately 120 μL) of the extracted specimen were added to the specimen wells of the test cassette, with a timer started immediately. Results were read between 5 and 10 minutes, and interpretation after 15 minutes was considered invalid to avoid non-specific reactions.

Result interpretation is as follows: a positive result for CPV is indicated by two distinct lines (control line C and CPV test line T); a positive result for CCV by two distinct lines (control line C and CCV test line C); a positive result for Giardia by two distinct lines (control line C and Giardia test line G); a negative result for all targets by only the control line C with no test lines visible; an invalid result when the control line C is not visible (regardless of test lines), requiring repetition with a new cassette and fresh specimen extract. For the reference method, Anigen Rapid CPV Ag Test Kit, Anigen Rapid CCV Ag Test Kit and Anigen Rapid Giardia Ag Test Kit (BioNote, Inc.) were used following their respective package inserts, with the same specimen extracts and interpretation criteria applied.

3. Results and Discussion

3.1 Results

3.1.1 Sensitivity and specificity

A total of 423 specimens were tested, with results for each target pathogen summarized in Table 1.

Table 1: In-House Clinical Study for CPV+CCV+Giardia Ag Combo Result.

Method		Commercial control test			Total Results
CPV+CCV+Giardia Ag Combo Rapid Test Cassette (Serum/Plasma) Evaluate Lot	CPV Ag	Results	Positive	Negative	
		Positive	45	0	45
		Negative	2	35	37
		Total Results	47	35	82
	CCV Ag	Results	Positive	Negative	
		Positive	54	3	57
		Negative	4	118	122
		Total Results	58	121	179
	Giardia	Results	Positive	Negative	
		Positive	39	5	44
		Negative	2	116	118
		Total Results	41	121	162

For CPV Ag:
Relative Sensitivity: 95.74% (95%CI*: 85.46%-99.48%)
Relative Specificity: 100.00% (95%CI*: 91.80%-100.00%)
Accuracy: 97.56% (95%CI*: 91.47%-99.70%)

For CCV Ag:
Relative Sensitivity: 93.10% (95%CI*: 83.27%-98.09%)
Relative Specificity: 97.52% (95%CI*: 92.93%-99.49%)
Accuracy: 96.09% (95%CI*: 92.11%-98.41%)
For Giardia
Relative Sensitivity: 95.12% (95%CI*: 83.47%-99.40%)
Relative Specificity: 95.87% (95%CI*: 90.62%-98.64%)
Accuracy: 95.68% (95%CI*: 91.30%-98.25%)

3.1.2 Cross-reactivity and Interference

To evaluate cross-reactivity, the combo test was challenged with specimens positive for other common canine enteric pathogens, including Salmonella spp., E. coli (enteropathogenic strains), and Cryptosporidium parvum (n=10 each). No cross-reactivity was observed, with all specimens testing negative for CPV, CCV and Giardia.

Interference testing was performed using specimens spiked with substances commonly present in feces/vomit, such as bile acids (0.5-2 mg/ml), hemoglobin (0.1-1 g/dl) and mucus (1-5% w/v). No significant interference was noted, with results consistent with unspiked controls.

3.1.3 Precision

Intra-assay precision was evaluated using 3 weak positive (antigen concentration near the limit of detection) and 3 strong positive specimens for each target, with 10 replicates per specimen. All replicates yielded consistent results (100% agreement).

Inter-assay precision was assessed across 3 days using the same specimens and 3 different kit lots. Agreement remained 100% for all targets, indicating robust reproducibility.

3.2 Discussion

3.2.1 Performance Characteristics

The CPV+CCV+Giardia Antigen Combo Rapid Test Cassette showed strong diagnostic performance across all three targets, with relative sensitivity, specificity and accuracy exceeding 93%-results comparable to or surpassing single-target antigen tests. For CPV, its high sensitivity (95.74%) and perfect specificity (100%) meet clinical needs: false negatives could delay critical care, while false positives might cause unnecessary isolation. The 2 false negatives involved fecal specimens with low viral loads (confirmed by qPCR), consistent with antigen tests' reduced sensitivity at low pathogen concentrations. CCV detection had slightly lower sensitivity (93.10%), possibly due to the enveloped virus' lower antigen stability in feces/vomit. Its 3 false positives, negative by reference kit but positive by the combo test, were confirmed as true negatives via PCR, suggesting potential cross-reactivity with non-pathogenic coronaviruses (needing further study).

Giardia detection (sensitivity 95.12%, specificity 95.87%) is notable given diagnostic challenges: traditional microscopy often misses low cyst counts, but the combo test matches immunofluorescence assay sensitivity. The 5 false positives might involve cross-reactivity with other protozoa (e.g., Entamoeba spp.), though not observed in cross-reactivity testing. Importantly, consistent performance in feces and vomit - with vomit easier to collect from acutely ill dogs - reduces repeated sampling, improving client compliance.

3.2.2 Limitations

Despite its strengths, the combo test has limitations: first, like all antigen tests, its sensitivity is dependent on pathogen load, with reduced performance in early or late-stage infections when antigen levels are low; thus, negative results in clinically suspect cases should be interpreted cautiously, with confirmation by PCR if necessary. Second, the test is qualitative and does not provide quantitative data on pathogen burden, which could be useful for monitoring treatment response - though qualitative results are sufficient for initial diagnosis. Additionally, storage and handling conditions are critical: specimens stored at 2 - 8℃ for more than 24 hours showed a 5% reduction in sensitivity, emphasizing the need for timely testing.

3.2.3 Comparison with Other Methods

Compared to single - target tests, the combo test reduces testing time by 66% (one test vs. three) and cost by approximately 40% (based on current market prices). This is particularly beneficial for resource-limited clinics.

Against PCR, the combo test sacrifices some sensitivity but offers speed and simplicity. PCR remains the gold standard for confirmation, but the combo test is superior for rapid screening in emergency settings.

4. Conclusion

The CPV+CCV+Giardia Antigen Combo Rapid Test Cassette is a reliable and practical tool for the simultaneous detection of CPV, CCV and Giardia antigens in canine feces and vomit specimens. Its high relative sensitivity, specificity and accuracy, combined with rapid results and ease of use, make it well-suited for point-of-care settings. By enabling timely identification of co-infections, this test supports targeted treatment and improved patient outcomes.

Future studies should evaluate its performance in larger, geographically diverse populations and against emerging CPV/CCV variants. Additionally, optimizing antigen extraction methods to enhance sensitivity for low-concentration specimens could further improve its clinical utility.

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