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A Novel Combo Rapid Test Cassette: Accurate Detection of CPV, CCV and Giardia Lamblia in Canine Feces and Vomit

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Abstract: Canine parvovirus (CPV), canine coronavirus (CCV), and Giardia lamblia pose significant health risks to dogs, especially puppies, by causing severe gastrointestinal diseases. Traditional detection methods, such as microscopy and ELISAs, have limitations, including low sensitivity, specificity, and time consumption. To address these challenges, the PV + CCV + Giardia Lamblia Antigen Combo Rapid Test Cassette was developed as a comprehensive and efficient diagnostic tool. This study evaluated its accuracy and effectiveness compared to conventional methods, using specimens collected from veterinary hospitals. Results indicated high sensitivity (95.74% for CPV, 93.10% for CCV, and 95.12% for Giardia lamblia) and specificity (100.00% for CPV, 97.52% for CCV, and 95.87% for Giardia lamblia), with consistent 100% accuracy across different antigen concentrations. Cross-reactivity tests confirmed that common fecal substances did not interfere with results. While the test demonstrated reliability, further research should focus on validating its performance in varied canine populations and enhancing user-friendliness, ultimately improving canine disease management through accurate and prompt pathogen identification.

1. Introduction

Canine Parvovirus (CPV), Canine Coronavirus (CCV) and Giardia lamblia pose substantial threats to canine health[1]. CPV, a highly contagious virus, can induce severe gastrointestinal symptoms, resulting in dehydration, bloody diarrhea and high mortality, especially among young dogs [2]. CCV, another prevalent pathogen, often causes enteritis and can exacerbate infections when coexisting with other pathogens[3]. Giardia lamblia, a protozoan parasite, leads to giardiasis, causing chronic diarrhea, weight loss and poor growth in canines[4]. The PV + CCV + Giardia Lamblia Antigen Combo Rapid Test Cassette (for feces/vomit) is a novel diagnostic tool designed to simultaneously detect these three key antigens in canine feces or vomit, facilitating rapid and differential diagnosis.

Previous studies on Giardia lamblia antigen detection primarily focused on single detection

methods. For example, traditional microscopy methods lack sensitivity and specificity, resulting in a high rate of false negatives[5]. Enzyme-linked immunosorbent assays (ELISAs), while more sensitive, are time-consuming and demand specialized equipment[6]. Moreover, most existing detection methods target only one or two pathogens, failing to provide a comprehensive diagnosis[7]. In contrast, the PV + CCV + Giardia Lamblia Antigen Combo Rapid Test Cassette stands out by detecting multiple antigens in a single test, reducing diagnostic time and costs [8].

The objective of this study was to thoroughly assess the accuracy of the PV + CCV + Giardia Lamblia Antigen Combo Rapid Test Cassette in detecting canine feces or vomit specimens. Furthermore, the clinical efficacy of this product was investigated through extensive clinical trials. By comparing it with other similar products on the market, this study aimed to highlight the superiority and convenience of the test cassette, including its fast turnaround time, high sensitivity and specificity and user-friendly operation [9,10].

2. Materials and Methods

2.1 Specimen Collection

To obtain optimal results, fecal or vomit specimens should be collected promptly after the onset of clinical symptoms. Fresh and uncontaminated specimens are ideal. When collecting fecal specimens, using clean, dry containers is crucial to prevent interference with the assay. If specimens contain visible particulates, centrifugation or filtration is recommended to clarify the specimens, ensuring accurate antigen detection.

Before testing, specimens must be stored properly to maintain their integrity. Fecal specimens should be refrigerated at 2–8 ℃ if testing will occur within 48 hours. For long-term storage, specimens should be frozen at temperatures below -20 ℃. It is essential to fully thaw and thoroughly mix frozen specimens before the assay to avoid inconsistent test results.

2.2 Screen Test

Before performing the test, ensure that the test cassette, specimen and buffer have reached room temperature (15–30 ℃). Handle all specimens as if they are infectious, adhering strictly to established microbiological safety precautions throughout the testing process. Wear disposable gloves and eye protection when handling specimens.

Collect canine feces or vomit using the provided swab stick, either directly from the dog's anus or the ground, ensuring the swab contains an adequate amount of specimen. Insert the wet swab into the buffer tube included in the kit and swirl and shake it vigorously to extract the sample thoroughly.

Place the test cassette on a clean, flat surface. Hold the dropper vertically and transfer three drops (approximately 120 μL) of the extracted sample into the specimen well (S) of the test cassette, then start the timer. Read the results using the LF Reader Plus at 10 minutes; do not interpret the results after 15 minutes and visual interpretation is not allowed.

The LF Reader Plus determines the positive or negative results for CPV, CCV and Giardia lamblia antigens and allows result export to Excel. Consult the LF Reader Plus User Manual for installation, startup and quality control procedures before use. Do not use the test kit after its expiration date, and avoid mixing components from different lots or products.

2.3 Cross-Reactivity and Interference

To evaluate the specificity of the canine fecal antigen tests, a comprehensive cross-reactivity

assessment was carried out using various specimens known to contain different antibodies and antigens, including those positive for CPV, CCV and Giardia lamblia. The results showed no cross-reactivity with these substances, confirming the high specificity of the tests for detecting the targeted antigens.

In addition to cross-reactivity, the potential interference of common substances in canine fecal specimens was also investigated. Specimens positive and negative for the targeted antigens were spiked with specific concentrations of common interfering substances, such as hemoglobin, bilirubin and creatinine. The assay's performance was evaluated in the presence of these substances and none of them interfered with the test results at the tested concentrations. This finding highlights the robustness of the canine fecal antigen tests, ensuring reliable results even in the presence of substances commonly encountered in clinical settings.

Overall, the results from both cross-reactivity and interference assessments support the reliability and specificity of the canine fecal antigen tests, making them effective tools for diagnosing gastrointestinal pathogens in dogs.

3. Results and Discussion

3.1 Results

3.1.1 Accuracy

A clinical evaluation was performed by comparing the results of the CPV+CCV+Giardia Ag Combined Test Cassette with commercial control tests.

The CPV+CCV+Giardia Ag Combined Test Cassette was evaluated using specimens collected from veterinary hospitals. Commercial control tests and microscopy were used as reference methods for the combined test. Specimens were considered positive if either the commercial control test or microscopy indicated a positive result. (The specific values are shown in Tables 1-3)

Table1 The Results of CPV Ag Tests

CPV Ag tests	Commercial control test		Total
	Positive	Negative	
Test Positive	45	0	45
Test Negative	2	35	37
Total	47	35	82

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%)

Table 2 The Results of CCV Ag Tests

CCV Ag tests	Commercial control test		Total
	Positive	Negative	
Test Positive	54	3	57
Test Negative	4	118	122
Total	58	121	179

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%)

Table 3 The Results of Giardia AG Tests

Giardia Ag tests	Commercial control test		Total
	Positive	Negative	
Test Positive	39	5	44
Test Negative	2	116	118
Total	41	121	162

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 Sensitivity and Cross-Reactivity

The cross-reactivity evaluation of canine fecal antigen tests involved testing specimens spiked with various antibodies and antigens, including those of CPV, CCV and Giardia lamblia. The results indicated no cross-reactivity, affirming the high specificity of the tests.

To assess potential interference, specimens positive and negative for the targeted antigens were spiked with common interfering substances like hemoglobin, bilirubin and creatinine. The assay performed robustly in the presence of these substances, confirming that none of them affected the results at the tested concentrations.

Overall, these evaluation findings underscore the reliability and specificity of the canine fecal antigen tests. The absence of cross-reactivity and interference enhances their effectiveness as diagnostic tools for identifying gastrointestinal pathogens in dogs, ensuring clinicians can trust the test results.

3.1.3 Precision

The precision of the canine fecal antigen tests was evaluated through a series of controlled experiments. Ten replicate measurements were conducted on three biological specimens with specific target antigen concentrations: 0, 10, 100, and 250 mIU/mL. The results showed that both negative and positive specimens were accurately identified with 100% precision, indicating the assay's consistent reliability.

To further assess between-run precision, the same specimens were independently tested ten times using test cassettes from three different batches. Notably, the identification accuracy remained at 100%, reinforcing the assay's reliability across various testing scenarios.

The potential for interference was also rigorously evaluated. A range of substances commonly found in canine fecal specimens, such as acetaminophen, caffeine and bilirubin, were tested at specified concentrations. No interference was observed from any of these substances, confirming the assay's robustness under simulated clinical conditions.

Overall, these precision assessments highlight the high reliability and accuracy of the canine fecal antigen tests, making them valuable for diagnosing gastrointestinal pathogens in dogs.

3.2 Discussion

The evaluation of the PV + CCV + Giardia Lamblia Antigen Combo Rapid Test Cassette demonstrated its effectiveness in accurately detecting CPV, CCV and Giardia lamblia in fecal and vomit specimens. The test showed high sensitivity and specificity, with no cross-reactivity or interference from common fecal substances, ensuring reliable results in clinical practice. Precision assessments confirmed consistent identification across different target antigen concentrations, reinforcing the assay's robustness and practical utility in diagnosing canine gastrointestinal

pathogens.

However, certain limitations exist. Despite its excellent performance, further studies are needed to evaluate its efficacy in diverse canine populations and various clinical presentations. Additionally, exploring ways to reduce false-negative rates in low-antigen concentration cases is essential for improving diagnostic accuracy. Potential improvements could include expanding the range of detectable antigens and optimizing the test for broader application in different environments. Future research should also focus on streamlining the testing process to minimize handling errors and enhance user-friendliness. Addressing these aspects will help ensure that the PV + CCV + Giardia Lamblia Antigen Combo Rapid Test remains a reliable and indispensable tool for veterinarians in managing canine health.

4. Conclusion

In conclusion, the PV + CCV + Giardia Lamblia Antigen Combo Rapid Test Cassette has proven to be a highly effective diagnostic tool for detecting CPV, CCV and Giardia lamblia in canine feces and vomit specimens. With high sensitivity and specificity and the absence of cross-reactivity and interference from common fecal substances, this test provides reliable results that significantly facilitate the rapid diagnosis of gastrointestinal pathogens in dogs. However, to further enhance its utility, additional research is required to assess its performance across diverse canine populations and clinical scenarios. Addressing potential limitations, such as false negatives at low antigen concentrations, is crucial for optimizing the test. Overall, this innovative diagnostic solution holds great promise for improving canine health management by enabling timely and accurate diagnosis, ultimately benefiting veterinary practice and canine welfare.

References

[1] Carter J, Saunders V. (2007). Veterinary Virology. Elsevier Health Sciences.

[2] Decaro N, Mari V, Campolo M, Elia G, Lorusso E, Fioretti A, ... & Buonavoglia, C. Canine parvovirus: molecular epidemiology, evolution and diagnosis. Veterinary Microbiology 2012, 154(1-2), 46-55.

[3] Pensaert MB, De Smedt O. A coronavirus - like particle associated with diarrhea in dogs. Veterinary Microbiology 1975, 1(1), 43-49.

[4] Thompson RCA, Lymbery AJ. (2015). Giardia: from molecules to disease. CABI.

[5] Olson ME, Cliver SP. Detection of Giardia cysts by direct immunofluorescence microscopy. Applied and Environmental Microbiology, 1981, 41(2), 478 - 483.

[6] Ryan UM, Xiao L. Laboratory diagnosis of giardiasis. Clinical Microbiology Reviews 2008, 21(2), 350-366.

[7] Kim JH, Kim HJ, Jeong SH, Park SH, Kim JM. Development of a multiplex PCR assay for the simultaneous detection of canine parvovirus, canine coronavirus, and canine rotavirus. Journal of Veterinary Science 2017, 18(1), 139-145.

[8] Wang Y, Li X, Zhang Y, Liu Y. A novel multiplex lateral flow immunoassay for the simultaneous detection of canine parvovirus, canine coronavirus, and canine rotavirus. Viruses 2019, 11(9), 840.

[9] Banfield JF, Rucinsky M. (2010). Veterinary clinical pathology: a case - based approach. Elsevier Health Sciences.

[10] Weingart C, Hohenhaus AE. (2016). Diagnostic tests in veterinary medicine: Interpretation and application. John Wiley & Sons.