

SENSITIVITY AND SPECIFICITY OF RAPIDE - CORONAVIRUS AG TEST KIT

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1. Abstract / Résumé

Rapide - Coronavirus Ag Test Kit is a new *in-vitro* immunochromatographic one step assay for detection of Coronavirus antigen in canine feces. Three (3) studies were conducted to check the sensitivity and specificity of this new diagnostic test:

In the first study, **Rapide - Coronavirus Ag Test Kit** was compared to PCR. Results showed a sensitivity of 93.1% (54/58) and a specificity of 97.5% (118/121).

In the second study, the field robustness of **Rapide - Coronavirus Ag Test Kit** was evaluated by sending 20 blinded fecal samples to three unrelated veterinary sites. The status of each sample was confirmed by PCR prior to the shipment to the study sites. Study investigators were requested to perform the test without knowing the status of each sample. Results show a 100% agreement between all study sites. The sensitivity was 100% (15/15) and the specificity was 100% (5/5).

In the last study, **Rapide - Coronavirus Ag Test Kit** was evaluated for potential cross-reactivity with Canine Distemper virus, Canine Parainfluenza Virus, Infectious Canine Hepatitis virus, Canine Parvovirus, *E. coli* sp., *Ascaris* homogenates, *Leptospira canicola* and *Giardia* homogenates. The results showed that **Rapide - Coronavirus Ag Test Kit** is highly specific for the canine Coronavirus antigen with no cross-reaction with any of the other common canine infectious pathogens.

Data from these studies show that **Rapide - Coronavirus Ag Test Kit** is highly sensitive and specific for detection of canine Coronavirus antigen in feces. Therefore, **Rapide - Coronavirus Ag Test Kit** is recommended to be adopted in animal clinics for diagnosis of canine Coronavirus infection.

Rapide - Coronavirus Ag Test Kit est un nouveau test d'immuno-migration *in-vitro* pour la détection de l'antigène du Coronavirus dans les fèces du chien. Trois (3) études étaient conduites pour vérifier la sensibilité et la spécificité de ce nouveau test de diagnostic:

Dans la première étude, **Rapide - Coronavirus Ag Test Kit** était comparé au test PCR. Les résultats montrent une sensibilité de 93.1% (54/58) et une spécificité de 97.5% (118/121).

Dans la deuxième étude, la fiabilité sur le terrain de **Rapide - Coronavirus Ag Test Kit** était évaluée en aveugle en envoyant 20 échantillons fécaux masqués à trois sites vétérinaires indépendants. Le statut de chaque échantillon était confirmé par PCR avant l'envoi vers les sites de l'étude. Les investigateurs conduisaient l'étude en aveugle sans connaître le statut de chaque échantillon. Les résultats montrent 100% de corrélation entre les sites de l'étude. La sensibilité est de 100% (15/15) et la spécificité est de 100% (5/5).

Dans la dernière étude, **Rapide - Coronavirus Ag Test Kit** était évalué pour des réactions croisées potentielles avec le virus canin de la maladie de Carré, le virus du parainfluenza canin, le virus de l'hépatite infectieuse canine, le parvovirus canin, *Leptospira canicola*, *E. coli* sp., homogenates d'*Ascaris*, et homogenates de *Giardia*. Les résultats montrent que **Rapide - Coronavirus Ag Test Kit** est extrêmement spécifique pour l'antigène du Coronavirus canin sans aucune réaction croisée avec d'autres pathogènes infectieux canins.

Les données de ces études montrent que **Rapide - Coronavirus Ag Test Kit** est extrêmement sensible et spécifique pour la détection de l'antigène du Coronavirus canin dans les fèces. Par conséquent **Rapide - Coronavirus Ag Test Kit** est recommandé en clinique vétérinaire pour le diagnostic de l'infection du Coronavirus canin.

2. Introduction

2.1 Introduction – Canine Coronavirus (CCV) is a virus that affects the intestinal tract of dogs. It causes a gastroenteritis similar to parvo. CCV is a highly contagious virus affecting not only puppies, but older dogs as well. The clinical signs of CCV vary from mild and undetectable to severe and fatal. Most common signs include: depression, fever, loss of appetite, vomiting, and diarrhea. The diarrhea can be watery, yellowish-orange in color, bloody, mucoid, and usually has an offensive odor. Sudden death and abortions sometimes occur. The duration of illness can be anywhere from 2-10 days. The most common route of infection is the contact with fecal materials containing the virus. Signs begin to show 1-5 days after exposure. The dog becomes a “carrier” for several weeks after recovery. The virus can live in the environment for several months.

2.2 Background - The **Rapide - Coronavirus Ag Test Kit** is an *in-vitro* immunochromatographic one step assay designed for qualitative determination of Canine Coronavirus antigen in feces.

2.3 Objectives - The primary objective was to determine the sensitivity and specificity of **Rapide - Coronavirus Ag Test Kit**.

3. Study design

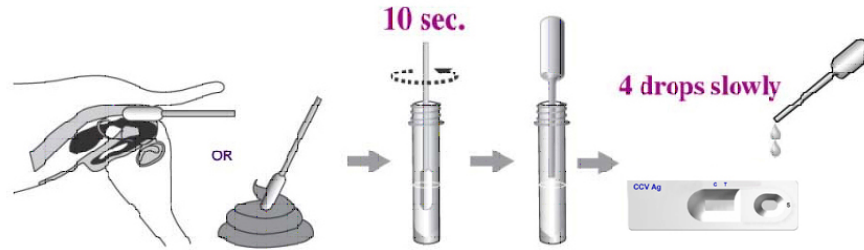
3.1 Study 1: This is a parallel comparative study. One hundred-seventy-nine (179) fecal samples from clinical cases were collected and tested by PCR, a laboratory reference standard test to confirm their positive or negative status for canine Coronavirus. All samples were then tested by **Rapide - Coronavirus Ag Test Kit** to evaluate the sensitivity and specificity compared to PCR.

3.2 Study 2: In this study, Five (15) positive and five (5) negative canine fecal samples were obtained from the University of Wisconsin. All samples were tested by PCR to confirm their positive or negative status. Samples were divided and shipped to three veterinary sites for blind testing using **Rapide - Coronavirus Ag Test Kit**.

3.3 Study 3: In this study, **Rapide - Coronavirus Ag Test Kit** was evaluated for potential cross-reactivity with other canine infectious pathogens which are commonly present in fecal materials: Canine Distemper virus, Canine Parainfluenza Virus, Infectious Canine Hepatitis virus, Canine Parvovirus, *E. coli* sp., *Ascaris homogenates*, *Leptospira canicola* and *Giardia homogenates*.

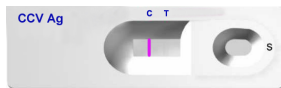
4. Materials & methods:

4.1 The **Rapide - Coronavirus Ag Test Kit** is an *in-vitro* immunochromatographic one step assay designed for qualitative determination of Canine Coronavirus antigen in canine feces. The study investigators were instructed to process the samples as indicated in the figure below:

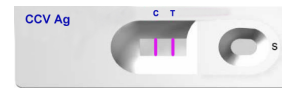


4.2 The Interpretation of the test results is conducted as follows:

Negative Result:



Positive Result:



5. Analysis

5.1 Sensitivity and specificity will be determined by standard calculation:

$$\text{Sensitivity}(\%) = 100 \times \frac{\text{No. of specimens with positive results by Rapid - Coronavirus Ag Test Kit}}{\text{No. of positive specimens confirmed by PCR}}$$

$$\text{Specificity}(\%) = 100 \times \frac{\text{No. of specimens with negative results by Rapid - Coronavirus Ag Test Kit}}{\text{No. of negative specimens confirmed by PCR}}$$

6. Results:

6.1 Study 1:

Sensitivity and Specificity		Gold Standard: PCR	
		Positive	Negative
Rapid - Coronavirus Ag Test Kit	Positive	54	3
	Negative	4	118
Sensitivity		93.1% (54/58)	
Specificity		97.5% (118/121)	

6.2 Study 2:

Sensitivity and Specificity			Gold Standard: PCR		Sensitivity	Specificity
			Positive	Negative		
Rapid - Coronavirus Ag Test Kit	Site A	Positive	15	0	100% (15/15)	100% (5/5)
		Negative	0	5		
	Site B	Positive	15	0	100% (15/15)	100% (5/5)
		Negative	0	5		
	Site C	Positive	15	0	100% (15/15)	100% (5/5)
		Negative	0	5		

6.3 Study 3:

Pathogens	Titer	Results
Canine distemper virus	$10^{4.5}$ EID ₅₀ /ml	Negative
Canine parvovirus	$10^{6.1}$ TCID ₅₀ /ml	Negative
Canine parainfluenza virus	$10^{5.5}$ TCID ₅₀ /ml	Negative
Canine hepatitis virus	$10^{4.5}$ TCID ₅₀ /ml	Negative
<i>Leptospira canicola</i>	OD 8.0 at 560nm	Negative
20% <i>Ascaris</i> homogenates	-	Negative
<i>Giardia</i> cyst suspension	-	Negative
<i>E. coli</i> spp.	$10^{8.0}$ CFU/ml	Negative

7. Discussion and conclusion:

These studies showed that **Rapide - Coronavirus Ag Test Kit** is highly sensitive and specific for the qualitative detection of Coronavirus antigen in feces. Using **Rapide - Coronavirus Ag Test Kit** is very simple, quick and it does not require any special equipments. Therefore, **Rapide - Coronavirus Ag Test Kit** is recommended to be adopted in animal clinics for diagnosis of canine Coronavirus infection.