



SENSITIVITY AND SPECIFICITY OF **RAPIDE** - DIROFILARIA AG TEST KIT

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1. Abstract / Résumé

Rapide - Dirofilaria Ag Test Kit is a new *in-vitro* immunochromatographic one step assay for detection of heartworm antigen in canine serum, plasma or whole blood. Four (4) studies were conducted to check the sensitivity and specificity of this new diagnostic test.

In the first study, **Rapide - Dirofilaria Ag Test Kit** showed a sensitivity of 94.4% (68/72) and a specificity of 100% (102/102).

In the second study, **Rapide - Dirofilaria Ag Test Kit** showed a sensitivity of 96.4% (53/55) and a specificity of 100% (54/54).

In the third study, the field robustness of **Rapide - Dirofilaria Ag Test Kit** was evaluated by sending 20 blinded serum samples to three unrelated veterinary sites. The status of each sample was confirmed 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 - Dirofilaria Ag Test Kit** was evaluated for potential cross-reactivity with canine parvovirus, canine distemper virus, canine parainfluenza virus, infectious canine hepatitis virus, porcine parvovirus, *Leptospira icterohaemorrhagiae*, *E. coli* spp., *Salmonella* spp., *Ascaris* homogenates, and *Giardia* homogenates. The results showed that **Rapide - Dirofilaria Ag Test Kit** is highly specific for the heartworm antigen with no cross-reaction with any of the other common canine infectious pathogens.

Data from these studies show that **Rapide - Dirofilaria Ag Test Kit** is highly sensitive and specific for detection of heartworm antigen in serum, plasma or whole blood. Therefore, **Rapide - Dirofilaria Ag Test Kit** is recommended to be adopted in animal clinics for diagnosis of heartworm infection.

Rapide - Dirofilaria Ag Test Kit est un nouveau test d'immuno-migration *in-vitro* pour la détection de l'antigène du ver du cœur dans le sérum, plasma ou sang total. Quatre (4) études étaient conduites pour vérifier la sensibilité et la spécificité de ce nouveau test de diagnostic.

Dans la première étude, **Rapide - Dirofilaria Ag Test Kit** montrait une sensibilité de 94.4% (68/72) et une spécificité de 100% (102/102).

Dans la deuxième étude, **Rapide - Dirofilaria Ag Test Kit** montrait une sensibilité de 96.4% (53/55) et une spécificité de 100% (54/54).

Dans la troisième étude, la fiabilité sur le terrain de **Rapide - Dirofilaria 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 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 - Dirofilaria Ag Test Kit** était évalué pour des réactions croisées potentielles avec le virus de la parvovirose canine, le virus canin de la maladie de Carré, le virus du parainfluenza canin, le virus de l'hépatite infectieuse canine, le parvovirus porcin, *Leptospira icterohaemorrhagiae*, *E. coli* spp., *Salmonella* spp. homogenates d'*Ascaris*, et homogenates de *Giardia*. Les



résultats montrent que **Rapide - Dirofilaria Ag Test Kit** est extrêmement spécifique pour l'antigène du ver du cœur sans aucune réaction croisée avec d'autres pathogènes infectieux canins.

Les données de ces études montrent que **Rapide - Dirofilaria Ag Test Kit** est extrêmement sensible et spécifique pour la détection de l'antigène du ver du cœur dans le sérum, plasma ou sang total. Par conséquent **Rapide - Dirofilaria Ag Test Kit** est recommandé en clinique vétérinaire pour le diagnostic de l'infection du ver du cœur.

2. Introduction

2.1 Introduction – Heartworm (*Dirofilaria immitis*) is a relatively large parasite that, in adulthood, lives in the heart and pulmonary arteries of an infected dog. Dogs acquire this infection when larval heartworms are transferred between infected and healthy dogs via mosquito bites. Geographic areas with high mosquito populations have severe heartworm problems.

2.2 Background - The **Rapide - Dirofilaria Ag Test Kit** is an *in-vitro* immunochromatographic one step assay designed for qualitative determination of heartworm antigen in serum, plasma or whole blood.

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

3. Study design

3.1 Study 1: One hundred-seventy-four (174) serum samples from clinical cases were collected. Seventy two (72) samples were confirmed positive and hundred-two (102) samples were negative. All samples were then tested by **Rapide - Dirofilaria Ag Test Kit** to evaluate the sensitivity and specificity.

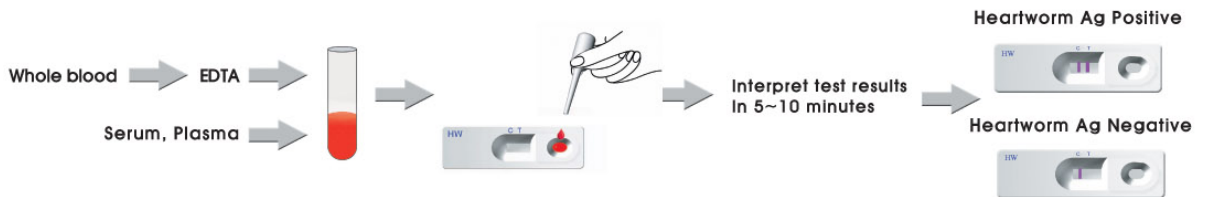
3.2 Study 2: This is a parallel comparative study. One hundred-fifty-five (109) serum samples from clinical cases were collected. Fifty-four (55) samples were positive and fifty-four (54) samples were negative. All samples were then tested by **Rapide - Dirofilaria Ag Test Kit** to evaluate the sensitivity and specificity.

3.3 Study 3: In this study, fifteen (15) positive and five (5) negative canine sera samples were obtained from clinical cases. Samples were divided and shipped to three veterinary sites for blind testing using **Rapide - Dirofilaria Ag Test Kit**.

3.4 Study 4: In this study, **Rapide - Dirofilaria Ag Test Kit** was evaluated for potential cross-reactivity with other canine infectious pathogens: canine parvovirus, canine distemper virus, canine parainfluenza virus, infectious canine hepatitis virus, porcine parvovirus, *Leptospira icterohaemorrhagiae*, *E. coli* spp., *Salmonella* spp., *Ascaris homogenates*, and *Giardia homogenates*.

4. Materials & methods:

4.1 The **Rapide - Dirofilaria Ag Test Kit** is an *in-vitro* immunochromatographic one step assay designed for qualitative determination of heartworm antigen in serum, plasma or whole blood. 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:

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

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

6. Results:

6.1 Study 1:

Sensitivity and Specificity		Necropsy	
		Positive	Negative
Rapide - Dirofilaria Ag Test Kit	Positive	68	0
	Negative	4	102
Sensitivity		94.4% (68/72)	
Specificity		100% (102/102)	

6.2 Study 2:

Sensitivity and Specificity		Necropsy	
		Positive	Negative
Rapide - Dirofilaria Ag Test Kit	Positive	53	0
	Negative	2	54
Sensitivity		96.4% (53/55)	
Specificity		100% (54/54)	



6.3 Study 3:

Sensitivity and Specificity			Necropsy		Sensitivity	Specificity
			Positive	Negative		
Rapide - Dirofilaria Ag Test Kit	Site A	Positive	5	0	100%	100%
		Negative	0	15	(15/15)	(5/5)
	Site B	Positive	5	0	100%	100%
		Negative	0	15	(15/15)	(5/5)
	Site C	Positive	5	0	100%	100%
		Negative	0	15	(15/15)	(5/5)

6.4 Study 4:

Pathogens	Titer	Results
Canine Parvo virus	$10^{4.0}$ TCID ₅₀ /ml	Negative
Canine Distemper virus	5×10^3 EID ₅₀ /ml	Negative
Canine Parainfluenza virus	$10^{6.0}$ TCID ₅₀ /ml	Negative
Infectious Canine Hepatitis virus	$10^{5.0}$ TCID ₅₀ /ml	Negative
Porcine Parvovirus	1,024 HAU	Negative
Leptospira icterohaemorrhagiae	OD 1.0	Negative
E. coli spp	$10^{8.0}$ CFU/ml	Negative
Salmonella spp.	$10^{8.0}$ CFU/ml	Negative
Ascaris homogenate	10%	Negative
Giardia homogenate	10%	Negative

7. Discussion and conclusion:

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