Comparative evaluation of Vcheck M Ehrlichia/Anaplasma with Real-time PCR

Key Words : Vcheck M, Ehrlichia, Anaplasma, Antibody rapid test, Real-time PCR, Sequencing

Introduction

Ehrlichia spp. and *Anaplasma* spp. are rickettsia bacteria that are transmitted by ticks to dogs and cats and are the causative agents of Ehrlichiosis and Anaplasmosis, respectively. They are important tick-borne diseases with a worldwide distribution. They can cause a variety of signs from none to fever and generalized achiness to possible fatality.
The veterinarian usually uses serologic tests and PCR tests to diagnose Ehrlichiosis & Anaplasmosis. In the past, PCR test samples had to be sent to an outside laboratory, but with the Vcheck M, PCR testing is possible in the veterinary clinic.

Purpose

The goal of this study is to evaluate the diagnostic sensitivity and specificity of the newly developed Vcheck M Ehrlichia/Anaplasma (POCT PCR kit) to laboratory-based real-time PCR.

Additionally, if there are discrepancies between the Vcheck M and lab-based real-time PCR results, sequencing will be requested to an external laboratory to confirm the final result.

Materials and Methods

SD Biosensor Inc., MDx R&D Department performed tests with Vcheck M and "P" kit (UK) with real-time PCR. Additionally, the sequencing tests were requested to the 'B' Laboratory (Korea) for confirmation.

The samples were a total of 35 canine whole blood checked positive by an antibody rapid test at a laboratory (Paraguay) and an animal hospital (Malaysia).

Results

The test results for the comparison of Vcheck M and lab-based real-time PCR are described in Tables 1, 2, and 3.

Conclusion

In this study, there were several discrepancies between the antibody rapid test and the two PCR tests. It is assumed that the dogs were infected with *Ehrlichia* or *Anaplasma* and then recovered.

In addition, discrepancies between Vcheck M and lab-based real-time PCR occurred in a total of 4 samples (Vcheck M positive, PCR negative). As a result of sequencing, these samples were confirmed to be positive.

Based on the results, it was confirmed that Vcheck M Ehrlichia/Anaplasma is superior to existing lab-based PCR in terms of not only convenience but also clinical performance.

Ehrlichia spp.		Reference method (Real-time PCR & seqeuncing)		
		Positive	Negative	Total
Vcheck M Ehrlichia/Anaplasma	Positive	18	0	18
	Negative	0	17	17
	Total	18	17	35
	Sensitivity	100% (18/18)		
	Specificity	100% (17/17)		

Ehrlichia spp.		Reference method (Real-time PCR & seqeuncing)		
		Positive	Negative	Total
Lab-based real-time PCR	Positive	15	0	15
	Negative	3	17	20
	Total	18	17	35
	Sensitivity	83.8% (15/18)		
	Specificity	100% (17/17)		

Table. 1 Sensitivity and specificity of Vcheck M Ehrlichia/Anaplasma and existing lab-based real-time PCR for *Ehrlichia* spp.

Anaplasma spp.		Reference method (Real-time PCR & seqeuncing)		
		Positive	Negative	Total
Vcheck M Ehrlichia/Anaplasma	Positive	3	0	3
	Negative	0	32	32
	Total	3	32	35
	Sensitivity	100% (3/3)		
	Specificity	100% (32/32)		

Anaplasma spp.		Reference method (Real-time PCR & seqeuncing)		
		Positive	Negative	Total
Lab-based real-time PCR	Positive	1	0	1
	Negative	2	32	34
	Total	3	32	35
	Sensitivity	33.3% (1/3)		
	Specificity	100% (32/32)		

Table. 2 Sensitivity and specificity of Vcheck M Ehrlichia/Anaplasma and existing lab-based real-time PCR for *Anaplasma* spp.

Sample No.	*Ehrlichia* spp.				*Anaplasma* spp.			
	Antibody Rapid	Vcheck M	Real-time PCR	Sequencing	Antibody Rapid	Vcheck M	Real-time PCR	Sequencing
22	*Ehrlichia* (+)	*Ehrlichia* (+)	(-)	*E. ewingii* (+)	(-)	(-)	(-)	(-)
24	*Ehrlichia* (+)	*Ehrlichia* (+)	(-)	*E. ewingii* (+)	(-)	*Anaplasma* (+)	(-)	*A. platys* (+)
27	*Ehrlichia* (+)	(-)	(-)	(-)	(-)	*Anaplasma* (+)	(-)	*A. platys* (+)
29	*Ehrlichia* (+)	*Ehrlichia* (+)	(-)	*E. canis* (+)	(-)	(-)	(-)	(-)

Table. 3 Sequencing results of discrepant samples between Vcheck M and existing lab-based real-time PCR