

Feb. 27, 2009

Evaluation of the Anigen Rapid Giardia Ag Test Kit

I submit this report to BioNote, Inc.



Yun young-min
College of Veterinary Medicine
Jeju National University

■ Cross reaction test against intestinal parasites and bacteria

1. Kit

[Anigen Rapid Giardia Ag Test Kit] Lot No. XXXXX

2. Intestinal parasites

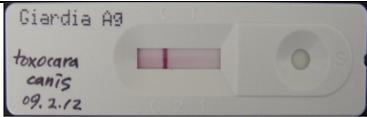
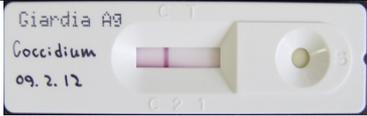
- 1) *Giardia lamblia*, *Coccidium*
(It was kept in college of veterinary medicine, Jeju national university.)
- 2) *Toxocara sp.*, *Anchylostoma sp.*
(It was extracted from infected dogs at the animal hospital of veterinary medicine, Jeju national university.)
- 3) *E. coli*, *Salmonella sp.*
(It was isolated and identified from the bacteriological lab of veterinary medicine, Jeju national university.)

3. Method

- 1) Counted the number of cysts and bacteria.
(When they were cysts, we diluted them with 10% suspension with PBS.)
- 2) Diluted them with assay diluents as same quantity.
- 3) Dropped them to the dropping site in the kit.
- 4) Observed the result within 10 minutes.

4. Result

All of the parasites(*Coccidium*) and bacteria were negative except the *Giardia lamblia*.

Name	Picture	Result
<i>Giardia lamblia</i>		Positive
<i>Toxocara canis</i>		Negative
<i>Coccidium</i>		Negative
<i>Anchylostoma canium</i>		Negative

<i>Salmonella</i>		Negative
<i>E. coli</i>		Negative

5. Conclusion

[Anigen Rapid Giardia Ag Test Kit] reacted to *Giardia lamblia* only.

■ Detection limit according to the number of cysts

1. Kit

[Anigen Rapid Giardia Ag Test Kit] Lot No. XXXXX

2. Giardia parasites

Giardia lamblia, kept in veterinary college, Jeju University, was inoculated to two dogs which were 60days old. It induced them to be infected with *Giardia lamblia*. We practiced *Giardia lamblia* test once a week and also did sensitivity of the kit according to the number of *Giardia* cysts.

3. Method

- 1) Floated *Giardia* cysts with assay diluents.
- 2) Counted them by the hemochamber.
- 3) Performed serial dilution.
(62.5, 125, 250, 500, 1000, 2000, 4000, 8000cyst/100ul.)
- 4) Dropped 100ul to each kit.
- 5) Observed band density after 10 minutes later.
- 6) Measured correlation.



A



B



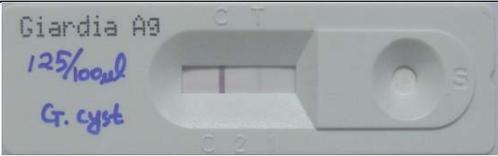
C



D

A: *Giardia* cyst in dilution of the kit B: *Giardia* cyst stained by iodine
C,D: *Giardia* cyst in hemochamber (X400)

4. Result

Concentration	Band density	Picture
62.5/100ul	-	 <p>Giardia Ag 62.5/100ul Gr. cyst</p>
125/100ul	1+, 10%	 <p>Giardia Ag 125/100ul Gr. cyst</p>
250/100ul	2+, 20%	 <p>Giardia Ag 250/100ul Gr. cyst</p>
500/100ul	2+, 30%	 <p>Giardia Ag 500/100ul Gr. cyst</p>
1000/100ul	2+, 40%	 <p>Giardia Ag 1000/100ul Gr. cyst</p>
2000/100ul	2+, 50%	 <p>Giardia Ag 2000/100ul Gr. cyst</p>
4000/100ul	3+, 65%	 <p>Giardia Ag 4000/100ul Gr. cyst</p>
8000/100ul	3+, 80%	 <p>Giardia Ag 8000/100ul Gr. cyst</p>

5. Conclusion

The detection limit of [Anigen Rapid Giardia Ag Test Kit] is 125cysts/100ul.

12. Comparison test with other commercial kit

1) Material

(1) Using reagent

A. Testing reagent : Anigen Rapid Giardia Ag Test kit (Korea)

Lot No.	Manufactured date	Expiration date
T 8002	2008. 4. 23.	

B. Compared reagent

Name	Manufacturing firm	Lot No.
SNAP Giardia	IDEXX	09511-ED416

C. Microscopic Examination

The fresh feces were collected from the rectum. We coated them on the slide glass slightly and observed them through the microscope at x100 or x400.

(2) Samples

Total 176 examined that suspected as diarrhea feces from five animal hospitals.

2) Method

Anigen Rapid Giardia Ag Test kit was used for diarrhea feces from five animal hospitals. Microscopic examination or IDEXX kit test was regarded as the gold standard at the same time. And we calculated specificity and sensitivity as following methods.

$$\text{Sensitivity} = \frac{\text{No. of samples with positive results by Anigen Rapid Giardia Ag Test kit}}{\text{No. of samples with positive results by IDEXX kits and microscope}} \times 100\%$$

$$\text{Specificity} = \frac{\text{No. of samples with negative results by Anigen Rapid Giardia Ag Test kit}}{\text{No. of samples with negative results by IDEXX kits and microscope}} \times 100\%$$

3) Examiner : Five animal hospitals (Reference, 12. Refer to confirmation of field test)

4) Result

Anigen Rapid Giardia Ag Test kit showed 100% of sensitivity and specificity. However, sensitivity and specificity of IDEXX are 95~96% and 99~100%. Therefore actual sensitivity and specificity of Anigen Rapid Giardia Ag Test kit are expected as over 95% and 99% (Lieb MS and Zajac AM. Giardiasis in dogs and cats. Veterinary Medicine. 1999:793-802, Hill SL *et al.* Prevalence of enteric zoonotic organ in cats. JAVMA. 2000;216(5):687-692).

Animal hospital name	Method	Total number examined	Difference test		Anigen Rapid Giardia Ag Test kit	
Hae-ma-ru animal hospital	IDEXX	52	9	43	9	43
Na-ra	IDEXX	9	3	6	3	6

animal hospital						
Hyun-dai animal hospital	Microscopic examination	15	0	15	0	15
Neul-pu-leun animal hospital	Microscopic examination	50	0	50	0	50
Heo animal hospital	Microscopic examination	50	0	50	0	50
Total		176	12	164	12	164

		IDEXX or Microscopic examination		
		Positive	Negative	Total
Anigen Rapid Giardia Ag Test kit	Positive	12	0	12
	Negative	0	164	164
	Total	12	164	176

(1) Sensitivity

$$\frac{12}{12} \times 100 = 100\%$$

(2) Specificity

$$\frac{164}{164} \times 100 = 100\%$$