Comparison of a *Histoplasma/Blastomyces* Lateral Flow Assay to Enzyme Immunoassay Using Dog and Cat Sera

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INTRODUCTION

- Quantitative enzyme immunoassays (EIAs) for *Histoplasma* and *Blastomyces* antigen are highly sensitive for the detection of fungal antigen to aid in the diagnosis of histoplasmosis and blastomycosis in dogs and cats.[1-3] These assays show nearly complete cross reactivity; therefore, both infections may be detected by a single antigen test.
- A serum lateral flow assay (LFA) was developed for human histoplasmosis, and this might be used in dogs and cats.
- Use of the LFA as a point-of-care assay in veterinary clinics would allow for more rapid diagnosis and earlier treatment of systemic mycoses
- The objective of this study was to <u>compare agreement between the *Histoplasma* antigen LFA and the quantitative EIAs for *Histoplasma* and *Blastomyces*,</u> using canine and feline serum.

METHODS

- Serum samples previously tested in either EIA, each tested in duplicate by LFA
    - 11 EIA-negative (22 LFA devices)
    - 21 EIA-positive (42 devices)
        - Ranged from 0.2 ng/mL to 19 ng/mL
    - Species distribution: 12 feline, 20 canine

- Serum was treated with EDTA and extracted supernatant was loaded onto the LFA device

- LFA devices were interpreted:
    - *Visually* by three qualified human evaluators (Fig. 1)
    - *Two automated readers* (Cube-Reader, opTricon GmbH, Germany) (Fig. 2).

- A **consensus RANN score** (ranging from 0-10 based on intensity of the test line compared to scorecard standards) was obtained by using two evaluator agreement. The samples were randomized and evaluators were blinded to EIA results.

- Cube reader T_{mean} also recorded and reported as positive or negative based on cutoff calculation

Fig. 1. LFA devices with RANN scores ranging from negative to high positive

Fig. 2. opTricon Cube-Reader placed over the LFA device

RESULTS

- Results comparison summarized in Table 1
- <u>Kappa agreement with EIA</u>:
    - Visual assessment **0.858**
    - Automated readers **0.861** and **0.760**
- EIA-negative samples with positive LFA results included:
    - Three devices with very low consensus RANN scores (1-2)
        - Two were also positive by both cube readers
        - One was positive by only one cube reader
    - All were canine samples
- EIA-positive samples with negative LFA results included:
    - One device with very low positive EIA result (0.5 ng/mL) and negative results by all LFA evaluations
    - Three additional devices with very low positive EIA results, negative by one cube reader
    - All were feline samples

Table 1. Comparison of *Histoplasma* or *Blastomyces* EIA results with LFA by visual assessment or opTricon Cube-Readers

EIA Result	LFA Visual Result		LFA Cube 1 Result		LFA Cube 2 Result	
	-	+	-	+	-	+
-	19	3	20	2	19	3
+	1	41	2	40	4	38

CONCLUSIONS

- *Good agreement between LFA and EIA*
- Uncommon false positive LFA results from some canine samples; very faint bands
    - also detected by automated readers (not evaluator error)
- Rare false negative LFA results from samples with low antigen concentration
    - Visual assessment outperformed automated readers
- **LFA may be a useful diagnostic tool for rapid serum diagnosis of histoplasmosis or blastomycosis in dogs and cats**

References:
1. Spector D, Legendre AM, Wheat J, et al. Antigen and antibody testing for the diagnosis of blastomycosis in dogs. *J Vet Intern Med* 2008;22:839-43.
2. Cook AK, Cunningham LY, Cowell AK, et al. Clinical evaluation of urine *Histoplasma capsulatum* antigen measurement in cats with suspected disseminated histoplasmosis. *J Feline Med Surg* 2012;14(8):512-5.
3. Cunningham L, Cook A, Hanzlicek A, et al. Sensitivity and specificity of *Histoplasma* antigen detection by enzyme immunoassay. J Am Anim Hosp Assoc. 2015;51(5):306-10.