MiraVista
VETERINARY DIAGNOSTICS

CLINICAL REVIEW

Invasive Fungal Infections NOT Invasive Diagnostic Investigations

(1,3)-β-D-Glucan Antigen Test in Dogs and Cats

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CLINICAL CHALLENGE:

Invasive fungal infections (IFI) often present a diagnostic challenge in dogs and cats. A few of the most notable include variable clinical presentations without pathognomonic clinical findings; disease in organ systems that require invasive procedures for sample collection; and slow fungal culture results that lack sensitivity.

More recently non-invasive tests to help support the diagnosis of an IFI have been developed. Some examples include the urine antigen enzyme immunoassay (EIA) for histoplasmosis or blastomycosis, the serum antigen latex agglutination test for cryptococcosis, and the serum antibody EIA or urine antigen EIA for coccidioidomycosis. Most of these tests are relatively specific to the fungal organism of interest. As such, there are countless fungal organisms that can cause an IFI for which specific non-invasive tests do not exist.

NON-INVASIVE TESTING OPTION

For many of the aforementioned organisms, the serum β-D-glucan (BDG) antigen test provides a non-invasive diagnostic option. Think of it as a pan-fungal antigen test, including infections with molds, yeasts, and fungal-like organisms such as oomycetes and *Pneumocystis spp* [1,2]. More specifically, diseases include CNS cryptococcosis, candidiasis, aspergillosis, hyalohyphomycosis, phaeohyphomycosis, pneumocystosis, and pythiosis [1-6]. There are some exceptions including blastomycosis and zygomycosis which have little or no BDG in the cell wall. Arguably this is not a significant detractor to the BDG antigen test as there are more specific tests available for blastomycosis (serum or urine antigen EIA). Treatment of pneumocystosis does not include antifungal drugs, and extra vigilance ruling-out this disease is recommended in clinical situations when pneumocystosis is more likely, for example in a young Miniature Dachshund or Cavalier King Charles Spaniel with pneumonia [7].

DATA IN DOGS AND CATS

The clinical performance of serum BDG was investigated in healthy dogs and cats and animals with histoplasmosis, coccidioidomycosis, systemic aspergillosis, and other systemic molds [8]. All IFIs were confirmed with cytology, histopathology, or culture. Overall serum BDG showed good sensitivity for histoplasmosis and systemic aspergillosis and low-moderate sensitivity for coccidioidomycosis (**Table 1**). Most notably 3/27 dogs with systemic aspergillosis and 3/27 dogs and cats with coccidioidomycosis tested negative for serum antigen, but tested positive for serum BDG. Moreover 4/4 dogs with other mold IFIs tested positive for serum BDG. As such, serum BDG might be a useful as an adjunct diagnostic test for many different IFIs.

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Table 1. Diagnostic performance of serum β-D-Glucan antigen test in dogs and cats with various confirmed IFIs, with other (non-fungal) disease, and healthy controls [8].

Diagnosis	Total Animals (N =256)	Overall Sensitivity (%)	Overall Specificity (%)	Antigen Specificity (%)
Histoplasmosis	27a	88		100 (urine)*
Coccidioidomycosis	27b	44		48*
Aspergillosis	27c	77		77*
Mold (other)	4d	100		
Non-fungal disease	112e		72	
Healthy controls	59f		88	

*Urine antigen testing= MiraVista *Histoplasma* urine antigen enzyme immunoassay; Antigen testing= MiraVista *Coccidioides* antigen enzyme immunoassay; Antigen testing= Platelia, antigen enzyme immunoassay. Dogs (Cats) in study: **a** Histoplasmosis 7(20), **b** Coccidioidomycosis 23(4), **c** Aspergillosis 27 dogs, **d** Mold (other) 4 dogs, **e** Non-fungal disease 99(13), **f** Healthy controls 55(4)

INDICATIONS FOR β-D-GLUCAN ANTIGEN TEST

While there remains a paucity of published data in veterinary medicine, based on data in other species, serum BDG antigen testing should be considered in the following clinical scenarios.

1. Where IFI is suspected and a definitive diagnosis cannot be obtained via cytopathology, histopathology or fungal culture [1].
2. Where IFI is suspected and testing for enzootic dimorphic fungi (*Histoplasma*, *Blastomyces*, *Cryptococcus*, *Coccidioides*) in the geographic area are negative [1].
3. Where invasive aspergillosis is suspected and the *Aspergillus* antigen EIA is negative [9,10].
4. For treatment monitoring of IFI when BDG antigen test is positive at baseline [2,4,11].
5. For treatment monitoring of pneumocystosis when BDG antigen test is positive at baseline [12].

CASE PRESENTATION:

Lady is an 11-year-old spayed, female cocker spaniel that was presented for tremoring and acting painful as evidenced by walking with a hunched posture. Physical examination revealed pain on lateral extension of the neck to the right and paraspinal palpation in the caudal thoracic region. The remainder of the physical examination was unremarkable. Routine lab work showed moderate hyperglobulinemia, mild hypoalbuminemia, moderate mature neutrophilia and monocytosis, collectively suggesting chronic inflammation.

Cervical and thoracic CT showed multiple lytic and proliferative bone lesions in the cervical and thoracic vertebrae (Figures 1 and 2) and a rib. Neoplasia or IFI was suspected. CT guided aspirate of the lesions showed mixed inflammation, but no evidence of neoplasia and no fungal organisms. The pet-owner was unable to pursue surgical biopsies. Due to suspicion of IFI, serum BDG test was submitted to MiraVista Diagnostics and was positive (125 pg/mL; normal< 60 pg/mL). Antifungal therapy, in addition to multi-modal treatment for pain, was instituted and clinical signs improved, but never completely resolved. Approximately 10 months later, Lady died of an unrelated

cause. In multiple vertebrae, necropsy and histopathology revealed granulomatous inflammation with aggregates of macrophages containing large yeasts. Fungal culture and/or molecular diagnostics were not performed to speciate the fungal organism.

In Lady's case a positive serum BDG test was supportive of the clinical suspicion of IFI. Since more invasive testing was not feasible, appropriate treatment was instituted. Monitoring serum BDG concentrations in addition to clinical signs and potentially repeat imaging could be useful for guiding treatment.

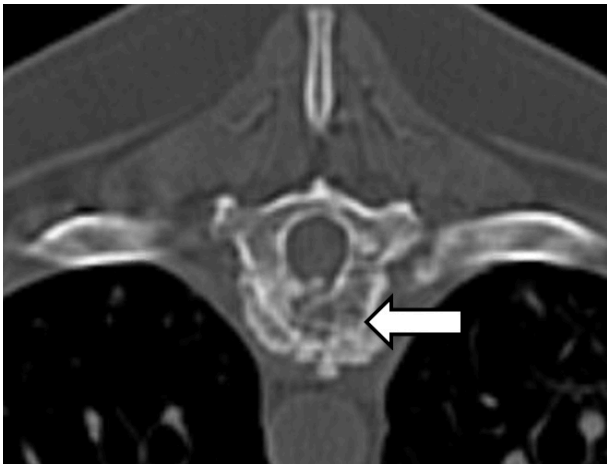

Figure 1. Transverse plane CT image of the osteolytic and proliferative lesion (white arrow) in the ninth thoracic vertebrae caused by an invasive IFI.

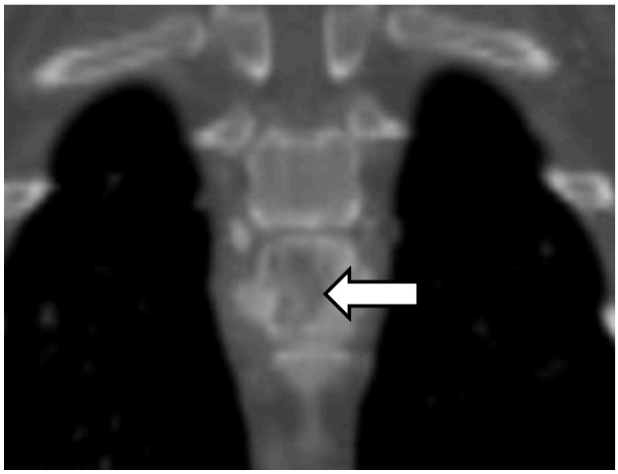

Figure 2. Dorsal plane CT image of the osteolytic and proliferative lesion (white arrow) in the ninth thoracic vertebrae caused by an IFI.

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(1,3)-β-D-GLUCAN ANTIGEN TEST at MIRAVISTA DIAGNOSTICS

Test Code
317

Clinical Significance
The Fungitell® assay measures the presence of (1→3)-β-D-Glucan in serum and CSF. Serum is the only specimen type cleared by the FDA for this assay. Performance characteristics of CSF have been validated by MiraVista Diagnostics.

Methodology
Colorimetric Fungitell® assay utilizing a (1→3)-β-D-Glucan -specific Limulus Amebocyte Lysate (LAL) reagent.

Specimen Collection
Collect serum specimens in serum separator or red top tube. Allow blood to clot for 30 minutes, then centrifuge. Pipette serum into a transport tube without interfering levels of (1→3)-β-D-Glucan. Most sterile polypropylene DNAase and RNAse free tubes are acceptable.

Minimum Specimen Requirements
Serum: 0.2 mL

Specimen Stability
Room Temperature: Not acceptable
Refrigerated: 5 days
Frozen: Indefinitely

Specimen Rejection
Lipemic, hemolyzed or icteric specimens may be rejected due to interference

Transport Temperature
Refrigerated/Frozen

Shipping
Ship on dry ice or frozen packs for next day service.
Monday – Friday delivery

Turnaround Time
Testing is performed Monday – Friday
Serum: 1-2 days

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