



CLINICAL DIAGNOSIS

Aspergillus Testing

If I suspect aspergillosis, should I order the antigen or the antibody test?

- That depends on the clinical presentation and suspected form of the disease.
- Screening for sinonasal aspergillosis (SNA) can begin with or include serologic testing for antibodies to *Aspergillus* by immunodiffusion (Test Code 324). The galactomannan antigen test performs poorly for this indication, as the sensitivity and specificity are low in dogs with SNA [1].
- If disseminated or pulmonary aspergillosis is suspected, then testing serum and urine in the galactomannan antigen test (Test Code 309) is the best option.

Where in the US does aspergillosis commonly occur?

- *Aspergillus* spores are ubiquitous in the environment; therefore, exposure occurs in all regions of the country. This differs from many other mycoses such as blastomycosis, coccidioidomycosis and histoplasmosis (with well-defined enzootic regions). Clinical disease from *Aspergillus* is more dependent on host factors, as many dogs have known or suspected immunosuppression.

What is the sensitivity and specificity of the agar gel immunodiffusion antibody test (AGID) for sinonasal aspergillosis?

- Previous studies evaluating AGID in dogs with SNA have shown sensitivity from 67-76% and specificity from 98-100%[1, 2]. The type of immunodiffusion reagent used in the assay may affect sensitivity, particularly if only *Aspergillus fumigatus* antigen is used. Both referenced studies used a similar AGID reagent that is included in the commercial MiraVista AGID assay, and it contains antigen derived from mycelial phase culture of *A. fumigatus*, *A. flavus*, and *A. niger*.

- Given the moderate sensitivity, a negative serology does NOT rule out SNA and further tests (diagnostic imaging, rhinoscopy, cytopathology, histopathology, culture, molecular testing) are indicated if the clinical presentation suggests SNA.
- Sinonasal *Penicillium* infection also occurs and may mimic the clinical presentation of SNA. The AGID test may be negative in cases of penicilliosis.
- The decision to treat for SNA should not be based solely on positive AGID results.

How does disseminated aspergillosis typically present in dogs?

- Many cases occur in young-middle aged, female, German Shepherd dogs and other long snout dogs, although other breeds may be affected. Disease may involve multiple organ systems and clinical features include diskospondylitis (vertebral pain, paraparesis, paraplegia), bone involvement (lameness, bone pain, fistulous tracts), pulmonary involvement, kidney involvement, CNS involvement, lymph node enlargement, uveitis/endophthalmitis, and nonspecific signs (fever, weight loss, anorexia, lethargy, vomiting, etc.). Body cavities may also be involved, and pericarditis, intra-abdominal infections, and pulmonary abscesses have been noted [3].

What is the best test for canine systemic (pulmonary or disseminated) aspergillosis?

- *Aspergillus* may be identified through culture of body fluids/specimens, and fungal hyphae may be visualized by cytology or histopathology. Antigen testing of body fluids (serum, urine, bronchial alveolar lavage specimens, cerebrospinal fluid) is a noninvasive way to assist in diagnosis of aspergillosis. Galactomannan antigen is also useful as a biomarker for fungal burden

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CLINICAL DIAGNOSIS

during therapy. PCR could potentially be useful, although studies are needed in dogs to characterize the sensitivity and specificity of PCR assays.

- Serum is likely the best specimen for galactomannan testing, as the serum result is typically slightly higher, and the sensitivity was marginally higher in one study compared to urine [4]. Rarely, the urine is positive, but the serum is negative. The highest sensitivity was achieved by testing both urine and serum.

How should galactomannan results be interpreted in dogs with suspected systemic aspergillosis?

- The galactomannan antigen test used at MiraVista is a commercial assay from Bio-Rad that was developed for use in humans with invasive fungal infection. Most of these humans are immunocompromised and require rapid diagnosis and antifungal therapy to improve their chance of survival. The assay cutoff is kept low (0.5 GMI) to preserve sensitivity; therefore, low positive results (0.5-2.0 GMI) may sometimes (8-14%) represent false positives.
- The galactomannan assay was evaluated in dogs in a 2012 study published in JVIM [4]. In 13 dogs with disseminated or pulmonary aspergillosis, the sensitivity and specificity were 92% and 86% (serum) and 88% and 92% (urine). The mean serum galactomannan was 7.44 GMI, and all dogs but one had results >7 GMI.

What are some causes of false positives in the galactomannan antigen test?

- Many other fungi express highly cross reactive galactomannan, including *Penicillium*, *Paecilomyces*, *Alternaria*, *Fusarium*, *Geotrichum*, and other molds. The galactomannan test is also useful for diagnosis and monitoring of systemic infections caused by these other fungi.
- Minor cross reactivity occurs between *Aspergillus* galactomannan and those of *Histoplasma* and *Blastomyces*. This means that high positive *Histoplasma*

or *Blastomyces* antigen results may result in low positive *Aspergillus* results. Aspergillosis rarely causes a false-positive *Histoplasma*, *Blastomyces* or *Coccidioides* antigen results.

- PlasmaLyte and other sodium gluconate containing fluids were previously shown to cause high false positives in the galactomannan assay. This may not be true at the current time [5], although no studies have been published to refute or confirm false positives from veterinary PlasmaLyte. It would be prudent to withdraw any sodium gluconate containing fluids for at least 48 hours before collecting specimens for galactomannan antigen testing.

**If you have questions, please call for a consult.
317-856-2681**

REFERENCE LIST:

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3. Day, M.J., *Canine Disseminated Aspergillosis*, in *Infectious Diseases of the Dog and Cat*, C. Greene, Editor. 2012, Elsevier: St Louis, MO. p. 662-666.
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