MiraVista
VETERINARY DIAGNOSTICS

FREQUENTLY ASKED QUESTIONS

Blastomyces Testing

QUESTION: If I suspect blastomycosis, should I order the antigen or the antibody test?

ANSWER: The MVista® *Blastomyces* Antigen Quantitative EIA (Test Code 316) is preferred for diagnosis of blastomycosis. We also offer the MVista® *Blastomyces* Canine IgG Antibody EIA (Test Code 330) which may be useful as a follow-up test in cases with suspected false negative or low positive antigen results. In cases with localized disease presentation (e.g., suspected ocular blastomycosis, solitary bone lesions, etc.), testing both antigen and antibody will likely improve sensitivity as we have observed more false negative antigen results in such cases. The antigen test may be used as a marker of therapeutic efficacy; therefore, obtaining a baseline urine antigen concentration is useful even in cytopathology or histopathology proven cases.

QUESTION: What is the preferred specimen for the antigen tests?

ANSWER: Urine is the preferred specimen. But 20% are positive only in serum (MiraVista)[1]. Consider sending both serum and urine and advising the lab to test the serum if the urine is negative. Typically, the urine antigen concentration will be higher than serum antigen concentration: In cases where the urine is above the limit of quantification (ALQ), serum may be monitored as a better marker for response to treatment, followed by urine once the serum is negative. CSF, BAL fluid and other body fluids may also be tested but the test has not been validated for testing tissue specimens.

QUESTION: What is the sensitivity and specificity of the antigen and antibody tests? (Table 1)

ANSWER: The sensitivity of antigen in urine is about 90% [2, 3]. Sensitivity in serum, based on experience in clinical testing, is slightly lower. False negative results are uncommon (5-10%) but do occur, and this would be more likely in cases with very early infection, mild disease, chronic disease or localized clinical signs. Antibody testing by EIA may be useful in cases with suspected false negative results if the organism has not been identified by cytology or histopathology. While the antigen test is entirely cross-reactive the antibody tests are more specific: Histoplasmosis and blastomycosis have overlapping endemic pattern of distribution and in those areas, antibody testing must be conducted for both histoplasmosis and blastomycosis.

The antigen is identical in histoplasmosis and blastomycosis. Otherwise the specificity of the antigen test is 98%[3]. Usually false positive results are low positives (<1 ng/mL). False positives in the moderate-high positive range are very rare. Dirty collection methods (e.g., cage floor collection, especially in exotic/zoo animals) may result in false positives results. Clean collection of the urine in a sterile container is recommended but cystocentesis may be required in some cases.

The MVista® *Blastomyces* Canine IgG Antibody EIA (Test Code 330) had 95% sensitivity in a study of dogs with blastomycosis[4]. This is much higher than the sensitivity (65%) of agar gel immunodiffusion (AGID)[4]. MiraVista has not developed a *Blastomyces* feline IgG antibody EIA but does test for Blastomyces antibody by AGID (Test Code 322). Concerns are unfounded over poor specificity due to long-term retention of IgG from previous exposure, as 95-100% specificity was observed in control dogs from the endemic region [5, 6].

Table 1: Sensitivity and specificity of Blastomyces antigen and antibody results in dogs

MVD test	Sensitivity % (pos/total)	Specificity % (neg/total)
Antigen-Urine	100 (21/21)	98 (40/41)
Antigen-Serum	100 (20/20)	100 (38/38)
IgG EIA Antibody	95 (19/20)	100 (38/38)
ID Antibody	55 (13/20)	100 (38/38)

Specificity was determined on healthy dogs or dogs with non-fungal pulmonary disease[4]. Cross-reactivity was observed in dogs with histoplasmosis: Antigen was detected in the urine in 6 of 8 (75%) dogs and serum was not tested. IgG antibodies were detected in the serum in 1 of 8 dogs (12%) and ID antibodies in none of 8.

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QUESTION: Do you have a fungal panel?

ANSWER: We do not but we have a strategy based on endemic pattern, Table 2.

Send both urine and serum specimens, requesting the primary test for your endemic area. Urine and serum should be tested for antigen as urine alone misses up to 20% of cases of blastomycosis. Request that if the primary test is negative to order the secondary tests.

Table 2. What Test to Order

Endemic	Primary	Secondary*
Blastomycosis	*Blastomyces* urine antigen (code 316)	*Blastomyces* serum antigen (code 316) *Blastomyces* IgG antibody (code 330): canines *Blastomyces* FID antibody (code 322): felines
Histoplasmosis	*Histoplasma* urine antigen (code 310)	*Histoplasma* serum antigen (code 310) *Histoplasma* IgG antibody (code 327): canines *Histoplasma* IgG antibody (code 328): felines *Histoplasma* FID antibody (code 321)
Both	*Blastomyces* urine antigen (code 316)	*Blastomyces* IgG antibody (code 330): canines *Blastomyces* FID (code 322): felines *Histoplasma* IgG antibody (code 327): canines *Histoplasma* IgG antibody (code 328): felines *Histoplasma* FID antibody (code 321)

*If the primary test is negative, all secondary tests listed should be ordered for the respective animal. For example, if Blastomyces urine antigen (code 316) is negative, then serum antigen (code 316) and IgG Antibody (330) for canines or serum antigen (code 316) and FID antibody (code 322) for felines should be ordered.

QUESTION: Is there cross reactivity between the *Blasto* and *Histo* antigen tests?

ANSWER: Yes, nearly complete (99%) cross-reactivity occurs in histoplasmosis and blastomycosis, and the discrepancies are in the low positive range[7]. Thus, **there is no need to order both antigen tests.** Less cross reactivity may be seen in other mycoses: *Blastomyces* antigen is positive in about 10% of cases of coccidioidomycosis and rarely in aspergillosis. Antibody testing may assist in resolving cross-reactivity.

QUESTION: I received a negative result for the *Blasto* antigen test. Can the antigen test be falsely negative?

ANSWER: Antigen concentration correlates with the severity of the infection, and results are almost always positive in moderately-severe or severe blastomycosis. Antigen tests may initially be negative in mild or localized cases and those presenting with brief duration of illness (1-2 weeks); therefore, a negative result does not exclude the diagnosis. In addition, nearly complete cross-reactivity occurs between antigen detection in histoplasmosis and blastomycosis, and current antigen tests cannot differentiate the two systemic fungal infections: There is no need to order testing for both antigens.

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QUESTION: If my practice is located an area that is endemic for both blastomycosis and histoplasmosis, which antigen test should I submit?

ANSWER: Due to cross reactivity, it is **unnecessary to test for both.** Blastomycosis is more likely if bone or skin lesions are present. For canine patients with GI signs, histoplasmosis would be more likely. Cats are much more likely to have histoplasmosis. *Histoplasma* antigen testing would be recommended in those cases.

Continue to order the test that was positive at baseline for monitoring response to treatment. Differentiation of the two infections is unnecessary, as treatment and monitoring are similar.

QUESTION: I received an antigen result that says "Positive, Below the Limit of Quantification (BLQ)". What does this mean?

ANSWER: A BLQ result is a very low positive (<0.2 ng/mL).

Results below 1 ng/mL may be truly positive or falsely positive. The result is likely to be truly positive if the clinical findings are consistent with blastomycosis. Testing for Blastomyces IgG antibodies (or *Blastomyces* and *Histoplasma* antibodies in areas were both are endemic) by EIA may help differentiate true from false-positive results. Repeat antigen testing is also recommended if the findings progress.

QUESTION: I am treating a patient for blastomycosis and the antigen concentration is not decreasing or is increasing. Is there a problem?

ANSWER: Yes, the antigen concentration declines with successful therapy. Some reasons for a failure to decrease include the following:

> Use of less effective antifungal: Non-FDA approved itraconazole (not compounded powder form of itraconazole) or fluconazole

> Subtherapeutic FDA approved itraconazole serum level (<2 mcg/mL). Obtain consultation if the antigen is not declining after 3 months of treatment.

QUESTION: How often should antigen concentration be determined?

ANSWER: Antigen should be tested in urine at 3-month intervals during treatment, at 6 and 12 months after stopping treatment and any time the clinical findings suggest recurrence. And if the urine antigen is "Above Limit of Quantification, ALQ" (>14.7ng/mL), serum should be tested instead. When the serum antigen is negative, resume monitoring urine antigen until negative.

QUESTION: Should the antigen test be negative at the end of therapy?

ANSWER: The patient should be treated until the antigen result is negative. Testing for antigenuria about every three months during and for 6-12 months after stopping therapy, and any time that clinical signs suggest recurrence, is recommended. Obtain consultation if the antigen is still positive after 12 months of treatment.

QUESTION: What are the criteria for discontinuing treatment?

ANSWER: Treat all patients for at least 6 months and until:

> The clinical findings have resolved

> The radiographic or other imaging findings have resolved or improved significantly and are thought to represent residual scarring (unchanged on at least 2 occasions)

> The antigen is negative

> If the antigen is not decreasing during the first 3 months of treatment, obtain consultation to determine if the treatment is effective (verify using FDA approved itraconazole)

> If the antigen remains positive, although lower concentrations after 12 months of treatment obtain consultation to determine whether to modify or stop treatment

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

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