

FREQUENTLY ASKED QUESTIONS

Blastomyces Testing

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

The MVista *Blastomyces* antigen test (Test Code 316) is the preferred screening test to aid in the initial diagnosis of blastomycosis. We also offer the canine *Blastomyces* IgG by EIA (Test Code 330) which may be useful as a follow-up test in cases with suspected false negative or equivocal 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 for any case.

What is the preferred specimen for the antigen tests?

Urine has shown the highest sensitivity in the antigen assay. Serum is also an acceptable specimen, although the sensitivity is slightly lower resulting in more false negative results. Typically the urine antigen concentration will be higher than serum antigen concentration; however, occasionally, the reverse is true. For this reason, some clinicians prefer to screen both urine and serum. CSF, BAL fluid and other body fluids may also be tested.

What are the sensitivity and specificity of the antigen and antibody tests?

- The *Blastomyces* urine antigen test has consistently shown around 90-95% sensitivity for acute disseminated blastomycosis in canine and human studies. The sensitivity when testing serum is slightly lower; therefore, urine is typically the preferred specimen. False negative results are uncommon but do occur, and this would be more likely in cases with very early infection, mild disease, chronic disease or localized clinical signs. We are currently studying the sensitivity of the antigen test in ocular blastomycosis. Antibody testing by EIA may be useful in cases with suspected false negative results (if the organism cannot be identified by cytology or histopathology).
- The specificity of the antigen test has been around 98-100% in studies. Usually false positive results are low positives (<1 ng/mL). False positives in the moderate-high positive range are very rare (excepting cross reactivity due to histoplasmosis or other fungal infections). Dirty collection methods of urine (e.g., cage floor collection [especially in exotic/zoo animals]) may result in low false positives or rarely false positives >1 ng/mL; therefore, clean collection of the urine in a sterile container or cystocentesis are recommended.
- The MVista canine IgG EIA had 95% sensitivity in a study of dogs with blastomycosis. This is much higher than the sensitivity of agar gel immunodiffusion antibody testing (AGID). 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.

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

Yes, nearly complete (99%) cross-reactivity occurs in histoplasmosis and blastomycosis, and the discrepancies are in the low positive range. Thus, **there is no need to order both tests**. Less cross reactivity may be seen in other mycoses: *Blastomyces* antigen is positive in about 20% of cases of coccidioidomycosis, 5% with aspergillosis and perhaps in sporotrichosis. Antibody testing may assist in resolving cross-reactivity.

I received a negative result for the Blasto antigen test. Does this mean that I should rule out blastomycosis in this patient?

- No, some patients with blastomycosis do not have detectable urine antigen. This is particularly true for animals with very early infection, mild disease, chronic disease or localized clinical signs. See more info on sensitivity above.
- It **is** much less likely that this is a Blasto case (especially if severe disseminated disease is present); therefore, other differential diagnoses should be pursued.

If my practice is located in an area that is considered to be endemic for both Blasto and Histo, which antigen test should I submit?

- Due to cross reactivity, it is typically not necessary or recommended to submit both antigen tests from the same patient. In states such as IN, IL, KY, TN and OH, the incidence of both blastomycosis and histoplasmosis is high; however, clinical blastomycosis seems to be more commonly observed in dogs. Blastomycosis is also more likely if bone lesions and/or skin lesions are observed in dogs. In these instances, submitting the Blasto antigen test would be recommended. For canine patients with GI signs, histoplasmosis would be more likely. Additionally, cats in the overlapping endemic areas are much more likely to have clinical histoplasmosis. Histo antigen testing would be recommended in those cases.
- If a baseline antigen result is obtained in either the Histo or Blasto antigen assays, the same assay should be utilized during therapeutic monitoring. The scale of reporting is different for the two assays (0.4 – 19.0 ng/mL for Histo; 0.2 – 14.7 ng/mL for Blasto).
- Differentiation of the two infections is often not necessary, as treatment and monitoring are very similar. If it is necessary to differentiate, antibody testing and/or definitive diagnosis by cytology/histopathology/culture should be undertaken.

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

- A BLQ result is a very low positive (<0.2 ng/mL). For further description of BLQ, see the BLQ information page.
- If this is a screening test for use in diagnosing blastomycosis, this result is somewhat equivocal and may represent a false positive. The antigen concentration is expected to generally correlate with severity of signs; therefore, it would be more likely that a BLQ result is a true positive for a case with localized disease (ocular signs only, bone/joint infection, etc.), very early infection, chronic long-standing infection or mild disease. Conversely, a case with severe disseminated disease is less likely to have a BLQ result. For equivocal low positive results, further efforts should be made to

identify the organism. Additional testing in the antibody EIA and/or repeat antigen testing are also recommended.

- If this result is obtained during treatment for blastomycosis, it indicates that the urine antigen concentration has decreased to *almost* negative. The most conservative approach to treatment indicates that antifungal therapy should be continued, and antigen reassessed every 1-2 months until a “negative, none detected” result is obtained.

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

Yes, the urine antigen concentration is expected to decline with successful therapy. Some reasons for a failure to decrease include the following:

- It may take some time for the concentration to decrease, so if you reassessed the antigen value within the first month of therapy, it may be too early to see a decrease. Typically it is acceptable to wait for at least 3 months until repeating the antigen test.
- If the initial antigen result was very high (above the limit of quantification; ALQ), this means that the concentration was >14.7 ng/mL. A follow-up result may also be ALQ, but since it is not quantifiable, it is not possible to see a decrease in concentration. If this happens, we can provide the optical density values for you compared to the daily cutoff in order to determine if a probable decrease in concentration has occurred.
- The antifungal therapy may not be effective. Most often, this is due to the use of compounded itraconazole. More information on itraconazole may be found on the itraconazole information page.

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

- Ideally, yes, the patient should be treated for blastomycosis until the urine antigen result is negative.
- A study using antigen levels as an aid in deciding when to stop therapy was published (Foy et al, JVIM 2014; 28:305-310). Among 27 dogs treated with fluconazole for an average of six months, antigen was detectable in the urine in 8 (30%) at discontinuation of treatment, but all at low levels (<1 ng/mL in 7 and 1.4 ng/mL in the 8th dog). Relapse occurred in 7 dogs (26%), 2 of which had a positive urine antigen at treatment discontinuation, including the dog with a result of 1.4 ng/mL. Relapse was associated with a rise in antigenuria in 5 of 7 dogs (71%), one of which occurred a month before the relapse. The authors recommended continuing therapy until:
 - clinical findings, including eye exam, resolved
 - thoracic radiographs were normal or stable
 - urinary antigen negative
- Continuing to monitor antigen concentration following discontinuation of antifungal therapy is beneficial, as rise in antigen indicates impending relapse.