

MVista® *Blastomyces dermatitidis* Antigen EIA

Test Code 314

MiraVista Diagnostics offers a new rapid method for the diagnosis of blastomycosis by antigen detection. Sensitivity is greatest in urine, but antigen also may be detected in other body fluids. Sensitivity and specificity are shown in the table. Antigen levels decline with therapy and increase with relapse, offering a method to monitor the effect of treatment and diagnose relapse.

Sensitivity and specificity of *Blastomyces dermatitidis* antigen assay.

Patient group	Number positive/total (%)
Pulmonary blastomycosis	13/13 (100)
Disseminated blastomycosis	26/29 (89.3)
Disseminated histoplasmosis	26/27 (96.3)
Coccidioidomycosis	0/9 (0)
Cryptococcosis, aspergillosis, candidiasis	3/166 (1.8)*

*2 cases of cryptococcosis and 1 case of aspergillosis

MVista® REPORTING AND EXPRESSION OF RESULTS

- All results are faxed to the referring lab.
- Critical Value notification is made to the referring lab.
- Results are reported as numeric values (EIA units) which are interpreted as positive or negative.
- Reference range is <1.0 EU.
- Concurrent prior results are reported with the follow-up specimen.

SPECIMEN REQUIREMENTS

- Specimen volume and preparation:
 - Acceptable samples include urine, serum, CSF, BALF or other sterile body fluid **excluding EDTA separated plasma**. 2.0 ml is required to permit confirmation testing and serial monitoring.
 - Serum should be separated from the clot.
- Refrigeration/cold packs are not required. Ambient shipping temperature is adequate.
- Shipment requirements: Leak-proof containers sent according to Federal Regulations.
- Specimen Labeling: Patient's name or ID# must be visible on the specimen.
- Shipping Address: 4444 Decatur Blvd., Suite 300, Indianapolis, IN 46241

LIMITATIONS OF THE METHOD

- EDTA is an interfering substance; therefore blood separated in an EDTA tube is an unacceptable sample. Heparinized blood, also called plasma, is acceptable.
- Heterophile antibodies and rheumatoid factor can cause positive interference.
- Cross reactions due to closely related antigenic epitopes are seen in patients with histoplasmosis, paracoccidioidomycosis, and penicilliosis.
- Rare cross reactions have been seen in patients with aspergillosis and cryptococcosis.

BILLING

- Referring facility will be billed.
- Prior positive specimens are tested concurrently at no extra charge.
- CPT Code 87449

MVista® *Blastomyces dermatitidis* Antigen EIA

Test Code 314

USES AND LIMITATIONS

- Aid in diagnosis of disseminated or acute pulmonary blastomycosis.
 - Urine is the most sensitive specimen type.
 - CSF or BALF improves sensitivity in meningitis or pulmonary blastomycosis.
- False-positive and false-negative results occur.
 - Antigen results must be correlated with clinical and other laboratory findings.
 - Repeat antigen if inconsistent with other findings or sole basis for diagnosis.
 - Recommend culture and serology if antigen sole basis for diagnosis.
 - Weak-positive results, 1-2 units, less likely to be reproducible, and should be verified by repeat testing.
 - A positive result in serum with a negative result in urine is rare and is cause for concern about a false-positive result caused by interfering substances, as described in the *Histoplasma* assay [1].
- Cross-reactions occur in histoplasmosis, paracoccidioidomycosis and penicilliosis.
 - Correct diagnosis can usually be distinguished by epidemiologic, clinical or other laboratory findings.
- Interassay variability averages 5-10%. To overcome interassay variability, using the test to monitor therapy or diagnose relapse, current and prior specimens are tested together.
- Monitoring therapy -- antigen declines with effective therapy.
 - Persistence suggests treatment failure.
 - Suggest testing every 3-6 months until negative.
 - The test is most sensitive if both serum and urine are monitored.
 - A marked change of more than 8 units in 4-6 weeks is unusual, as noted in histoplasmosis [2], and is cause for concern about some mix up in specimens, and warrants repeat testing.
- Diagnosing relapse -- antigen increases at least 2 units in 90% of cases.
 - Suggest testing every 3-6 months and at the time of suspected relapse.
 - The test is most sensitive if both serum and urine are monitored.

Assay Methodology. The assay is a sandwich enzyme immunoassay (EIA) using polyclonal antibodies to *Blastomyces dermatitidis* [1]. Microtiter wells are coated with rabbit anti-*Blastomyces* antibodies, incubated and washed. EIA blocking reagent is added to each well, incubated and washed. Next, the test specimen is added to each well, incubated and washed. The wells are incubated with enzyme-linked rabbit anti-*Blastomyces* IgG and washed. Finally, *Blastomyces* antigen is measured by adding the enzyme substrate to each well. The plate is incubated and washed. Color development is stopped by the addition of H₂SO₄ and the plate is read on an ELISA reader. Results are divided by 1.5 X the mean value of the cutoff calibrators and expressed as EIA units (EU). Specimens yielding a result of 1 unit or higher are retested the next work day.

Interpretive guidelines are provided with the test results (sample report upon request). Of note, negative results do not exclude blastomycosis, occurring in at least 14% of cases. Positive results support a diagnosis but should be verified by retesting and supported by other tests including serology and culture. In all cases, the antigen results must be used in conjunction with clinical findings and other laboratory results, as the antigen test may be either falsely negative or falsely positive. A key advantage of the test, however, is its good sensitivity and ability to provide a rapid diagnosis.

Follow-up specimens. Antigen levels decline with therapy and increase with relapse, offering a method to monitor the effect of treatment and diagnose relapse. Follow-up specimens obtained at least 14 days after the last prior specimen are tested simultaneously with prior specimens in the same assay to assess the course of antigen clearance in all patients with prior positive results, or with known blastomycosis, based on information provided by the referring physician or laboratory. A computer database is automatically reviewed when each day's specimens are entered into the daily test program to determine if that patient had been tested previously. Simultaneous testing is required because interassay variability

precludes direct comparison of results between assays. Results for both the current and prior specimen are reported back to the ordering laboratory, along with guidelines for interpretation of changes in antigen values.

MVista® *Blastomyces dermatitidis* Antigen EIA

Test Code 314

Reporting of results. The assay is performed daily Monday through Friday. Negative results, positive results > 4 EU or follow-up results with no increase, are faxed by midnight on the day of testing. For positive samples, preliminary results are available by calling MiraVista Diagnostics after 4PM EST on the day of testing; however, final results are not reported until they are verified the following work day. Clients are notified of critical values after final results are generated. A rare specimen (0.2%) is classified as equivocal because reproducible results cannot be obtained after testing on three occasions.

About half of specimens are processed through intermediary laboratories rather than shipped directly to MiraVista Diagnostics, delaying the turn-around time by at least one day. Shipping directly to MiraVista or calling the intermediary laboratory 48 to 72 hours after the specimen was shipped can avoid such delays. The ordering laboratory, physician, or physician's representative can call MiraVista directly to determine if the specimen has been tested, but MiraVista is only permitted to release the result to the intermediary laboratory unless the physician's name has been provided on the requisition.

Assay sensitivity and specificity. Specimens from patients with proven blastomycosis and controls were tested blindly and results were calculated without knowledge of the source of the specimen or patient identity. Results were then sorted by diagnosis into five groups: pulmonary blastomycosis, disseminated blastomycosis, cross reacting mycoses, other mycoses, and normal donors. The sensitivity was 89.3% in cases of disseminated blastomycosis and 100% of pulmonary cases. A cross reactive antigen was present in specimens from patients with histoplasmosis, paracoccidioidomycosis, and penicilliosis marneffeii. Specificity was 94.3% in patients with other mycoses and 98.8% in normal controls.

Assay precision and reproducibility. In an evaluation of results in patients and controls tested in consecutive assays, results were reproducible in 95.7% of cases, and the actual result of the initial and repeat value correlated closely ($R^2=0.976$).

Quality control of new reagents and assay materials. Following CLIA and CAP regulations, all new lots of assay reagents and supplies require parallel testing with reagents/supplies currently in use. All QC results are documented and kept on file for two years. The parallel testing method includes a panel of matrix materials, serial dilutions of known concentrations of patient samples and possible cross-reactive substances that are tested with both the new lot and the current lot. To be acceptable, the new lot must not have $\geq 20\%$ mean difference from the current lot.

- 1) The laboratory runs tests on patient specimens concurrently with the controls of graded reactivity plus a negative control and control values must not vary from those seen in previous assays before the assay is considered valid and results are released.
- 2) A general or technical supervisor must release all assays before results are reported.
- 3) Any problems or trends seen are immediately discussed with a supervisor. Final decision on the validity of an assay is at the discretion of the director.
- 4) Any assay problems are documented.
- 5) All control values are evaluated as part of the monthly and semi-annual QC reports. Any unusual shifts or trends are investigated.



4444 Decatur Blvd., Suite 300
Indianapolis, IN 46241
1-866-MiraVista
Phone 317-856-2681
FAX: 317-856-3685
www.miravistalabs.com

L. Joseph Wheat, M.D., Director

Clinical Consults: x452
Result Inquires: x450

Current licensure available on our website

MVista® *Blastomyces dermatitidis* Antigen EIA

Test Code 314

Reference List

1. Durkin M, Witt J, LeMonte A, Wheat B, and Connolly P. Antigen Assay with the Potential To Aid in Diagnosis of Blastomycosis. *J Clin Microbiol* 2004; 42:4873-5.
2. Wheat LJ. Current diagnosis of histoplasmosis. *Trends Microbiol* 2003; 11:488-94.
3. Wheat LJ, Connolly P, Durkin M et al. False-positive *Histoplasma* antigenemia caused by antithymocyte globulin antibodies. *Transpl Infect Dis* 2004; 6:23-7.