



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 Inquiries: x450

Current licensure available on our website

MVista® *Histoplasma capsulatum* Quantitative Antigen EIA

Test Code = 310

MiraVista Diagnostics offers a rapid method to aid in the diagnosis of histoplasmosis by antigen detection [1]. Measurement of antigen in both urine and serum offers the highest sensitivity for diagnosis of acute pulmonary or disseminated histoplasmosis. Testing bronchoalveolar lavage may improve the sensitivity for diagnosis of pulmonary histoplasmosis [2, 3], and CSF for diagnosis of meningitis. For monitoring therapy, testing both urine and serum offers the highest sensitivity for assessing improvement and diagnosing relapse. Cross reactivity occurs in blastomycosis[4], coccidioidomycosis [5], paracoccidioidomycosis [4] and penicilliosis marneffeii [4], and rarely in aspergillosis [6].

NOTE: Beginning February 16, 2009, serum and other fluids with blood contamination will first be treated with EDTA at 100° C for six minutes to eliminate inhibition caused by antibodies, which will improve sensitivity and increase antigen concentration.

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 ng/ml, by extrapolation from a calibration curve, which are interpreted as positive, critical values or negative.

MVista® INTERPRETATION OF RESULTS

Results of Initial Testing

Reference Range: None Detected

Result	Interpretation	Comment
None detected	Negative	Antigen not detected
<0.6-3.9 ng/ml	Positive, low	Results reported as <0.6 ng/ml are positive but below the lowest calibrator and cannot be quantified.
4.0-19.9 ng/ml	Positive, moderate	Quantitation is most accurate in this area of the calibration curve.
20.0->39 ng/ml	Positive, high	Results >39 ng/ml are above the highest calibrator and cannot be quantified.

Guidelines for comparing current specimen results with previous results¹

(Only results from identical specimen types can be compared)

For Low-Moderate Positives (< 20 ng/ml)	Interpretation	For High Positives (≥ 20 ng/ml)
Change in Ag		Change in Ag
> 3 ng/ml increase	Probable treatment failure/relapse	> 15% increase
≤ 3 ng/ml decrease	Possible treatment failure	≤ 15% decrease
> 3 ng/ml decrease	Probable treatment response	> 15% decrease

¹Suggest monitoring antigenemia and antigenuria, see above comment about EDTA-heat treatment of serum.

SPECIMEN REQUIREMENTS

- Acceptable specimen types: Urine, serum or plasma separated from the clot, CSF, BAL or other sterile body fluid, stored and shipped ambient. Storage or shipment refrigerated or frozen will not adversely affect the test.
- Shipping requirements: Leak-proof containers sent according to Federal Regulations to the address noted above.
- Specimen Labeling: Patient's name or ID# must be visible on the specimen.

LIMITATIONS OF THE METHOD

- Anti-rabbit or heterophile antibodies and rheumatoid factor can cause positive interference.
- Cross reactions are noted in the first paragraph above.
- Sputolysin and NaOH are both interfering substances.

BILLING

- Referring facility will be billed.
- CPT Code 87385

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GUIDELINES FOR USE

- As an aid in rapid diagnosis of disseminated or acute pulmonary histoplasmosis.
 - Testing both urine and serum offers the highest sensitivity, as some patients may have negative results in one but positive results in the other specimen type.
 - Serum, plasma and other specimens that appear to contain blood are treated with EDTA/heat to allow dissociation of immune complexes. This pre-treatment can increase sensitivity by 95% in specimens that previously tested negative.
 - Serum is particularly useful for monitoring therapy (see below), and should be tested if initially positive.
 - CSF or BALF improves sensitivity in meningitis or pulmonary histoplasmosis.
- False-positive and false-negative results occur.
 - Antigen results must be correlated with clinical and other laboratory findings.
 - Repeat the antigen testing if the result is inconsistent with other findings or the sole basis for diagnosis.
 - Culture and serology are recommended if antigen is the sole basis for diagnosis.
 - Weak-positive results, <0.6 to 3.9 ng/ml, are 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 anti-rabbit or heterophile antibodies [7].
- Cross-reactions occur in blastomycosis, coccidioidomycosis, African histoplasmosis, paracoccidioidomycosis and penicilliosis. Correct diagnosis can usually be distinguished by epidemiologic, clinical or other laboratory findings.
- Monitoring therapy: Antigen declines with effective therapy.
 - Failure of antigen to decline by at least 20% during the first month of therapy and 20% during subsequent 3-month intervals suggests treatment failure.
 - Suggest testing after one month of therapy and then every 3-4 months until negative.
 - Antigen declines more rapidly in serum than urine, and antigen concentration in serum is less likely to be affected by hydration status than is the concentration in urine. If the baseline serum is positive, it should be monitored until negative, and then urine should be monitored until negative.
- Diagnosing relapse: Antigen increases at the time of relapse in up to 90% of cases. The magnitude of change suggestive of relapse varies over the wide range of antigen concentrations. A 3 unit increase is concerning for relapse in specimens with results < 20 ng/ml compared to a 15% increase in specimens with results > 20 ng/ml.
 - Suggest testing every 3 months during therapy and at the time of suspected relapse.
 - Most sensitive if both serum and urine are tested at the time of suspected relapse.
- Results from other labs may not correlate with those from MiraVista - be sure of where the test was performed.

Assay Methodology. The assay is a sandwich enzyme immunoassay (EIA) using polyclonal antibodies to *Histoplasma capsulatum* [8]. The assay was modified in 2004 to reduce false-positive results caused by anti-rabbit antibodies and improve sensitivity [9]; in 2007 to incorporate quantitation [10] and in 2009 to include pre-treatment of serum, plasma and hemolyzed specimens with EDTA/heat [11].

Microtiter wells are coated with rabbit anti-*Histoplasma* antibodies, incubated and washed. EIA blocking reagent is added, incubated and washed. Next, the test specimen is added to each well, incubated and washed. The wells are incubated with enzyme-linked rabbit anti- *Histoplasma* antibody and washed. Finally, *Histoplasma* 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 H₂ SO₄ and the plate is read on an ELISA reader. Results are extrapolated off of a standard curve (ng/ml). Specimens yielding a result above the cutoff are regarded as positive.

Interpretive guidelines are provided with the test results (sample report upon request). Of note, although the sensitivity of the MVista® *Histoplasma* antigen assay is over 95% in disseminated histoplasmosis, and nearly 90% in acute pulmonary histoplasmosis, if both urine and serum are tested, negative results do not exclude histoplasmosis.

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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. Because of increased assay to assay reproducibility, **in the third-generation quantitative assay, prior specimens are no longer tested concurrently with follow-up specimens.**

Reporting of results. The assay is performed daily Monday through Friday. Negative results and positive results > 3.9 ng/ml are faxed by midnight on the day of testing. For positive samples from <0.6-3.9 ng/ml, 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.

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. Antigen can be detected in the urine of 98-100% of individuals with AIDS and disseminated histoplasmosis [10; 12]. Sensitivity in patients with other underlying diseases complicated by disseminated histoplasmosis and in subacute pulmonary histoplasmosis have not yet been established in the quantitative assay [10], but are expected to be higher than using the older method. The sensitivity in acute pulmonary histoplasmosis is nearly 90% if both urine and serum are tested

A cross reactive antigen was present in specimens from patients with blastomycosis, coccidioidomycosis, paracoccidioidomycosis, African histoplasmosis and penicilliosis marneffeii. False-positive results also have been observed in organ transplant patients who have been treated with rabbit anti-thymocyte globulin [7], which has been largely overcome in the later generation assays [9; 13].

Assay precision and reproducibility. In an evaluation of results in patients and controls tested in consecutive assays, results were reproducible in over 98% of cases, and the actual result of the initial and repeat value correlated closely ($R^2 = 0.9811$).

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 a minimum of 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.

- 1) The laboratory runs tests on patient specimens concurrently with the controls of graded reactivity, a standard curve plus a negative control. Control values and the slope of the standard curve must fall within acceptable ranges and not vary from those seen in previous assays before the assay is considered valid and results are released. A general or technical supervisor releases all assays before results are reported.
- 2) Any problems or trends seen are immediately discussed with a supervisor. Final decision on the validity of an assay is based upon company guidelines following CAP and CLIA guidelines. Any assay problems are documented.
- 3) All control values are evaluated as part of the monthly and semi-annual QC reports. Any unusual shifts or trends are investigated.

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1. Wheat LJ. Improvements in diagnosis of histoplasmosis. *Expert Opin Biol Ther* 2006; 6:1207-21.
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