



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® Azole Microbiological Assay Itraconazole test code: 312

BACKGROUND: The blood concentrations of the azole antifungal agents may be affected by medications or conditions that induce or inhibit cytochrome P450, and with the exception of fluconazole, and can vary greatly from subject to subject [1]. Furthermore, low concentrations may lead to treatment failure. Whether high concentrations cause toxicity is less clear, however. Therapeutic drug monitoring has been recommended when using itraconazole [2,3]. Bioassays measure the antifungal effect of the patient’s blood on a *Candida* strain susceptible to the azoles, and may give falsely-high values if the patient is receiving other antifungal agents or calcineurin inhibitors. Of note is that studies rigorously establishing the drug levels associated with treatment response or toxicity or showing that drug level monitoring with dose adjustment based upon drug level have not been conducted. **The proposed target concentrations are based upon limited data suggesting an association between blood level and response, and are thus provided as mere guidelines. Formal studies of drug level monitoring with dose adjustment based upon level would be needed to prove the utility of drug level monitoring and to establish the levels predictive of response or toxicity. Accordingly, recommendations in this document should be regarded only as guidelines, and do not established a standard of care.**

Itraconazole. Median trough concentration in immunosuppressed patients receiving itraconazole capsules 400 mg daily for one week for prophylaxis was 0.31 µg/ml, ranging from undetectable to about 0.8 µg/ml, and were below 0.5 µg/ml in 62% of patients [2]. Somewhat higher concentrations are achieved using the oral solution (0.66 µg/ml), but 20% remain below 0.5 µg/ml. Trough concentrations below 0.5 µg/ml were associated with failure of prophylaxis [3]. Levels associated with treatment failure for systemic or invasive mycoses have not been determined, but random levels > 2.0 µg/ml are recommended [4]. In patients with AIDS receiving itraconazole capsules 200 mg twice daily for histoplasmosis, random concentrations exceeded 2 µg/ml in 85% of cases [8]. Itraconazole half-life is long (>24 hr), and levels vary little over a 12 to 24-hour dosing interval, supporting the feasibility of using random levels if the time of administration is not known. In patients receiving itraconazole capsules 200 mg twice daily, concentrations exceed 10 µg/ml in 10-20% of cases, which would appear to be unnecessary.

Drug	Random
Itraconazole	≥ 1.0 µg/ml

REPORTING AND EXPRESSION OF RESULTS

- All results are faxed to the referring lab.
- Results are reported as numeric values (µg/ml) which are interpreted as none detected, below the lowest point on the standard curve, subtherapeutic range, therapeutic range or above the highest point on the standard curve.
- Concentrations below the proposed target levels may be subtherapeutic, but therapeutic targets have not been validated through clinical trials.

SPECIMEN REQUIREMENTS

- Specimen volume and preparation:
 - Acceptable samples include serum, plasma, and CSF
 - 2.0 ml is preferred, 0.5 ml is required.
 - Serum should be separated from the clot.
 - FREEZE on day of collection and remain frozen during shipment.

CAP 7182293
 CLIA 15D0996282
 Medicare Part B 190460
 NPI 1588600456

CA COS800133
 FL L800017215
 MD 1077
 NY 8036

NJ 25ms00007800
 PA 028127
 RI 00360



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® Azole Microbiological Assay Itraconazole test code: 312

- Recommended supplementary information:
 - Timing of the specimen following the last dose of the medication
 - Name of co-administered antifungal agent
- Refrigeration/cold packs are required.
- 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

- Co-administration of an echinocandin or amphotericin B may cause falsely high estimates of azole concentration. Testing by other methods is suggested in patients receiving echinocandins. No effect has been seen with calcineurin inhibitors, however.
- Therapeutic and toxic concentrations have not been accurately defined in clinical trials correlating drug concentration with outcome.
- Concentration determined on a single specimen may not reflect future concentrations because of changes in adherence, drug dosage, route of administration, absorption, or receipt of other medications affecting absorption or metabolism of the azole.

BILLING

- Referring facility will be billed. MiraVista does not bill patients or insurance.
 - CPT Code is 80299.
-

CAP 7182293
CLIA 15D0996282
Medicare Part B 190460
NPI 1588600456

CA COS800133
FL L800017215
MD 1077
NY 8036

NJ 25ms00007800
PA 028127
RI 00360



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® Azole Microbiological Assay Itraconazole test code: 312

MVista® GUIDELINES FOR USE

- To be used as an aid in monitoring antifungal therapy
- Guidelines have not been rigorously proven by clinical trial
- Co-administration of an echinocandin may cause falsely high estimates of azole concentration. Testing by other methods is suggested in patients receiving echinocandins. No effect has been seen with calcineurin inhibitors, however.
- Follow-up testing may be necessary if there are changes in treatment, including starting or stopping interacting medications, or suspicion of treatment failure

Methodology. The antifungal drug present in a patient specimen will produce a clearing zone on agar seeded with *Candida sp.* By preparing a standard curve of zone measurements, the antifungal level in the patient specimen can be quantified.

Follow-up specimens. Follow-up specimens should be tested to assess the effect of a modification in dosage, route of administration, drug interaction caused by a medication that alters absorption or metabolism of the azole.

Reporting of results. The assay is performed twice weekly, on Tuesday and Thursday, with results released the following day.

Assay sensitivity. The lower detection limit of the Itraconazole assay is 0.31 µg/ml.

Assay specificity. The bioassay uses a strain of *Candida kefyr* that is susceptible to itraconazole. Thus, the presence of this azole in the test specimen will cause a zone of inhibition, yielding a detectable concentration of the antifungal agent in the test specimen.

This strain of *Candida kefyr* used as the indicator organism also is susceptible to caspofungin and amphotericin B. Thus, the concurrent administration of the azole with amphotericin B and/or caspofungin will interfere with interpretation of the results, since amphotericin B and caspofungin also will inhibit growth of indicator organism.

Precision and reproducibility.

Precision was assessed by testing five aliquots of three specimens spanning the detection range (µg/ml):

Aliquot	Specimen 1 (BC # 8)	Specimen 2 (BC # 9)	Specimen 3 (BC #10)
1	6.95	0.51	1.48
2	6.26	0.43	1.67
3	8.22	0.43	1.63
4	7.22	0.47	1.55
5	6.61	0.43	1.48
Mean ± 1 standard deviation	7.052 ± 0.746	0.454 ± 0.036	1.562 ± 0.086
Coefficient of variation (CV), as SD÷mean	0.106	0.079	0.055



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® Azole Microbiological Assay Itraconazole test code: 312

Assay to assay **Reproducibility** was examined by testing five specimens on two different days (µg/ml):

Test date	Specimen 1	Specimen 2	Specimen 3	Specimen 4	Specimen 5
1	<0.125	0.16	0.20	0.28	2.52
2	<0.125	0.20	0.28	0.36	2.98
Mean day 1 & 2	<0.125	0.18	0.24	0.32	2.75
Difference day 1 vs. day 2	0	0.04	0.08	0.08	0.46
CV, as difference ÷ mean	0	0.222	0.333	0.250	0.167

Operator to Operator Reproducibility was assessed by two technologists testing five specimens (µg/ml):

Technologist	Specimen 1	Specimen 2	Specimen 3	Specimen 4	Specimen 5
1	<0.125	0.16	0.16	0.28	2.79
2	<0.125	0.16	0.20	0.32	2.52
Mean	<0.125	0.16	0.18	0.30	2.655
Difference	0	0	0.04	0.04	0.27
CV	0	0	0.222	0.133	0.102

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.

- 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 releases 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.

Reference List

1. Leveque D, Nivoix Y, Jehl F, and Herbrecht R. Clinical pharmacokinetics of voriconazole. Int J Antimicrob Agents 2006; 27:274-84.
2. Glasmacher A, Hahn C, Molitor E, Marklein G, Sauerbruch T, and Schmidt-Wolf IG. Itraconazole through concentrations in antifungal prophylaxis with six different dosing regimens using hydroxypropyl-beta-cyclodextrin oral solution or coated-pellet capsules. Mycoses 1999; 42:591-600.
3. Glasmacher A, Hahn C, Leutner C et al. Breakthrough invasive fungal infections in neutropenic patients after prophylaxis with itraconazole. Mycoses 1999; 42:443-51.
4. Wheat J, Hafner R, Korzun AH et al. Itraconazole treatment of disseminated histoplasmosis in patients with the acquired immunodeficiency syndrome. AIDS Clinical Trial Group. Am J Med 1995; 98:336-42.

CAP 7182293
 CLIA 15D0996282
 Medicare Part B 190460
 NPI 1588600456

CA COS800133
 FL L800017215
 MD 1077
 NY 8036

NJ 25ms00007800
 PA 028127
 RI 00360