



Improved Serologic Diagnosis Coccidioidomycosis

Introduction.

Delays in the diagnosis of coccidioidomycosis are associated with coccidioidomycosis related cost totaling \$589,053 [1]. A study assessing the knowledge of clinicians found many were unsure how to use serologic tests for diagnosis of coccidioidomycosis [2], contributing to the cost of care [1]. The authors emphasized the importance of providing training to physicians in areas where coccidioidomycosis is endemic on how to diagnose coccidioidomycosis and use the diagnostic tests appropriately.

Khan and colleagues reported in 2018, results of a study performed in 2013 at three laboratories in highly endemic areas of Arizona and California [3]. Specimens from 150 patients with clinical findings of coccidioidomycosis and positive ID or CF and 50 non-endemic controls were tested at three laboratories. Interlaboratory agreement was 90% and 89% for the IMMY EIA, respectively and 67% and 80.5% for the Meridian EIA [3]. Sensitivity was 68% and specificity 99.3% for the IMMY EIA and 72.4% and 91.3% for the Meridian EIA. The authors noted that the “sample size lacks sufficient statistical power to draw firm conclusions regarding sensitivity and specificity”. Also, the use of healthy subjects from non-endemic areas as controls prevents accurate assessment of specificity. They concluded “the lower overall sensitivity of both the EIA test kits... merits further study and research into more sensitive diagnostic test for this disease” and that “further study of the sensitivity and specificity of *Coccidioides* EIA test kits is warranted”.

Malo and colleagues [4, 5] reported findings that address some of the limitations of the Khan study [3]. Between 2014-2017 103 subjects with progressive disseminated or pulmonary coccidioidomycosis and 170 controls were tested in the MiraVista, IMMY and Meridian *Coccidioides* IgG and IgM antibody EIAs. A proven diagnosis was based on positive culture or pathology and a probable diagnosis on clinical

findings and serologic findings by ID or CF. Controls included healthy subjects from Tucson, Arizona and Bakersfield, California and 22 subjects enrolled in the prospective study that did not have coccidioidomycosis (clinical controls) and 50 healthy subjects from Miami, Florida (non-endemic controls). *Coccidioides* IgG and IgM antibody testing was performed at MiraVista Diagnostics.

Results.

The MiraVista EIA was more sensitive (88.3%) than ID (60.2%) or CF (53.2%), table 1. EIA results are compared in figure 1. Specificity was 89.2% by EIA and 98.8% by ID. Sensitivity of the MiraVista EIA was 88.3%, IMMY EIA 59.2%, and Meridian EIA 71.8%, table 2. Specificity was determined by testing 112 endemic control specimens. Specificity was 89.2% in the MiraVista EIA, 86.4% in the IMMY EIA, and 87% in the Meridian EIA. Indeterminant results were included with negative results in determination of specificity. Sensitivity was greatest in the MiraVista EIA and specificity was not significantly different than the MiraVista, IMMY or Meridian EIAs.

Three hundred and seventy-three specimens from cases and controls were tested on different days. Results of 10 EU and above are positive. Results are depicted in figure 2. IgG results were reproducible in 372 (99.7%) and IgM in 364 (97.6%). R^2 was 0.984, $P < 0.00014$ for IgG and 0.993, $P < 0.0001$ for IgM [4]. CF titers and IgG EU correlated weakly: correlation coefficient 0.209 (weak correlation) and the P value 0.102 [6].

Discussion.

Sensitivity of the MiraVista EIA was greater than ID, CF or the IMMY and Meridian EIAs, $P < 0.001$. Indeterminant IgG EIA results were most common in the IMMY EIA (17.5%) and least (5.8%) in the MiraVista EIA, table 2. Specificity for IgG or IgM for healthy endemic controls was no different, 90%



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for MiraVista, 93.7% for IMMY and 95.5% for Meridian (P = 0.055). Indeterminant results occurred in 15.5% of endemic controls in the MiraVista, 8.9% in the IMMY and 1.1% in the Meridian EIA. Specificity for IgG or IgM was 100% in non-endemic controls [4].

Weakly positive IgG results in the MiraVista EIA (10-19.9 EU) occurred 25.2% of cases and 8.1% of controls. They may represent current or prior coccidioidomycosis. Follow-up testing 3-4 weeks later should be considered if the diagnosis is uncertain: IgG and IgM concentration may increase in subjects with recent infection but not in past infection.

CF is usually performed to monitor changes in antibody levels if the ID test is positive [7]. The *Coccidioides* Serology Laboratory at the University of California Davis stores

specimens to compare the prior and current specimen in the same assay and considers a > 2-fold differences as significant. They reported that titers cleared slowly during treatment in subjects with uncomplicated pulmonary coccidioidomycosis (PUC), chronic pulmonary coccidioidomycosis (PCC) and disseminated coccidioidomycosis (DC) [8]. Median improvement rates were 91 days/dilution for PUC, 112 days/dilution for PCC and 136 days/dilution for DC subjects. The MiraVista EIA incorporates a standard curve between 0 and 80 EU which permits reproducible quantification (figure 2) without testing the current and prior specimens together. Changes in IgG EU in the MiraVista EIA may provide a better marker for monitoring treatment than CF titers. MiraVista is seeking FDA clearance to allow use of its EIA in CLIA certified laboratories for diagnosis and monitoring treatment of coccidioidomycosis.

Table 1. Comparison of MiraVista anti-*Coccidioides* antibody EIAs, ID and CF

Classification	MiraVista IgG (103)	MiraVista IgM (103)	MiraVista IgG or IgM [2]	ID (102) [1]	CF (62) [1]
Positive	87.4%	61.2%	88.3%	60.2%	53.2%
Indeterminant	4.8%	9.7%	5.8%	Not applicable	Not applicable
Negative	7.8%	29.1%	5.8%	39.8%	46.8%
[1] P <0.001					

Table 2. Comparison of IgG or IgM anti-*Coccidioides* EIAs

Cases (N = 103)	MiraVista [1]	Meridian [1]	IMMY [1]
Positive	88.3%	71.8%	59.2%
Indeterminant	5.8%	12.6%	17.5%
Negative	5.8%	15.5%	23.3%
[1] P <0.001			

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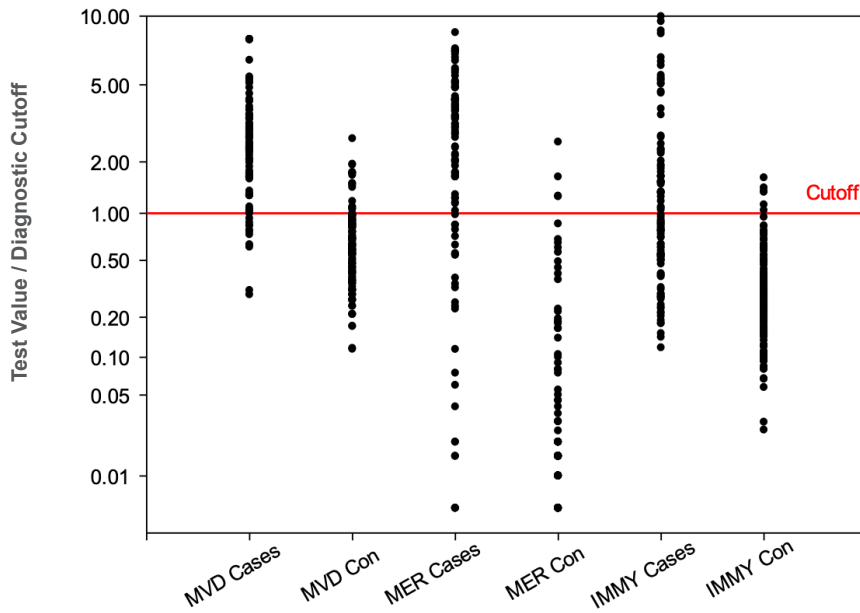
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Figure 1. Comparison of MiraVista IgG EUs in cases and controls (Con)



Legend. EUs or ODs transformed into multiple of cutoff: MiraVista 10 EU, IMMY 1.5 EU [Omega Cocci AB EIA Test Kit, IMMY.com] and Meridian Bioscience [Premier *Coccidioides*, OD>0.200]

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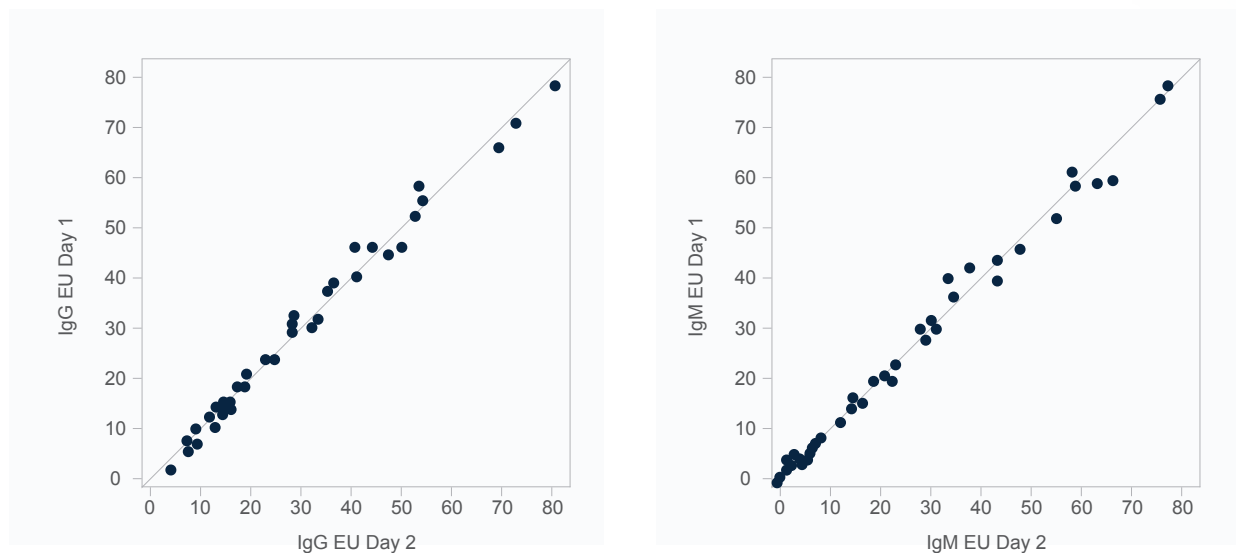
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Figure 2. Inter-assay reproducibility in the MiraVista IgG and IgM EIAs



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