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Comparative Evaluation of Commercial Premier EIA and Microimmunodiffusion and Complement Fixation Tests for *Coccidioides immitis* Antibodies

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A total of 409 serum and cerebrospinal fluid specimens from human subjects with proven coccidioidomycosis, with other infections, or with no apparent illness were tested for antibodies to *Coccidioides immitis* by the Premier EIA (Meridian Diagnostics, Inc., Cincinnati, Ohio), which tests for immunoglobulin G (IgG) and IgM responses to coccidioidal antigens, and by the conventional complement fixation (CF) or immunodiffusion (ID) assays for antibodies corresponding to those detected by the tube precipitin (TP) or CF tests. Of the 409 specimens, 47 were from persons with confirmed coccidioidomycosis and all were positive for *C. immitis* antibodies in IDCF tests and enzyme immunoassays (EIAs) for both IgG and IgM. The EIA for detecting both IgG and IgM antibodies proved to be sensitive for detecting coccidioidomycosis case sera positive by the IDCF, IDTP, and CF tests. Maximal sensitivity for diagnosing coccidioidomycosis is dependent upon detection of both IgG and IgM antibodies in the EIA. The EIA, however, was not absolutely specific, since some sera from patients with confirmed blastomycosis and some from patients with noncoccidioidal disease produced false-positive reactions.

Diagnosis of *Coccidioides immitis* infections is usually based on the cultural isolation of the etiologic agent, the histopathologic detection of the endosporulating spherules of the fungus in clinical materials, and/or the demonstration of the presence of specific coccidioidal antibodies in clinical fluids by complement fixation (CF), immunodiffusion (ID) CF (IDCF), tube precipitin (TP), or IDTP tests (1, 3). The present investigation represents a comparative evaluation of the Premier EIA (Meridian Diagnostics, Inc., Cincinnati, Ohio) and the ID and CF tests for detecting *C. immitis* antibody.

MATERIALS AND METHODS

Specimens. A total of 409 serum and cerebrospinal fluid specimens were tested. These specimens were obtained from subjects with culturally, histologically, and/or serologically proven coccidioidomycosis ($n = 47$); from persons with pulmonary illness without any evidence of coccidioidomycosis ($n = 345$); from persons with no apparent illness ($n = 3$); and from persons with aspergillosis ($n = 3$), blastomycosis ($n = 5$), histoplasmosis ($n = 3$), or human immunodeficiency virus infection ($n = 3$). All specimens were tested by microimmunodiffusion for the presence of IDCF antibody and by enzyme immunoassay (EIA) for *C. immitis* immunoglobulin G (IgG) and IgM antibodies. Fourteen of the 47 specimens were from patients with early coccidioidomycosis and were IDTP positive.

EIA. EIAs were performed by using the reagents and following the instructions provided by the manufacturer. The procedural details included the dilution of serum (1:441) and cerebrospinal fluid (1:21) specimens with an aliquot of diluent provided with the kit. One hundred microliters of each specimen was added to duplicate microtiter plate wells precoated with coccidioidal antigens and incubated at room temperature (24°C) for 30 min. Following incubation, the contents of the microwells were discarded and the wells were washed three times with buffer provided with the kit. One hundred microliters of conjugated anti-human IgG or IgM was then pipetted into each of the duplicate microtiter plates. The conjugates were allowed to react with the contents of their respective wells for 30 min at 25°C. The unbound contents from the microwells were then discarded, and the microwells' contents were washed three times with the buffer. One hundred microliters of substrate was then added to each microwell, and the plates were incubated for 10 min at 25°C. The substrate reaction was terminated by the addition of stop solution (2 drops per microwell). Microwells of positive and negative controls for the detection of IgG and IgM antibodies were treated as described above. Absorbance values were read, by using a dual-wavelength Dynatech Model 730 reader, at 450 and 630 nm. According to the manufacturer, a positive specimen is one that yields an A_{450}/A_{630} of 0.2 or greater. Any specimen yielding readings of 0.15 and <0.2 was considered inconclusive, and a repeat test was performed in order to exclude a technical error during the performance of the test. Sera with inconclusive values gave reproducible values in repeat tests. If the repeat test read <0.2, the specimen was regarded as negative. Specimens giving an initial reading of <0.1 were regarded as negative for IgG and/or IgM antibody.

ID and CF tests. The IDCF, standardized Centers for Disease Control CF, and IDTP tests were performed and interpreted according to protocols described elsewhere (1, 2).

Reagents. Reference anti-*C. immitis* serum and coccidioidin for the IDCF test were obtained from Adams Scientific (West Warwick, R.I.) and ID Laboratories (London, Ontario, Canada). The CF reagents were prepared and the tests were performed at the Centers for Disease Control and Prevention (Atlanta, Ga.). The IDTP reference antiserum and antigen were provided by D. Pappagianis (3).

RESULTS

The results of our evaluation of the 47 specimens obtained from persons with culturally, histopathologically, and/or serologically proven coccidioidomycosis are summarized in Table 1. All the proven coccidioidomycosis case specimens were positive by the EIA for both IgG and IgM antibodies. If a single EIA was performed for IgG or IgM alone, sensitivity diminished. Forty-three (92%) of these 47 specimens were positive for IgG, and 36 (77%) were positive for IgM. All the specimens demonstrated IDCF precipitins.

The results of tests with heterologous sera from persons with aspergillosis, blastomycosis, or histoplasmosis or with suspected pulmonary disease without laboratory evidence of coccidioidomycosis, as well as the results of tests with sera from human immunodeficiency virus-infected persons and appar-

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TABLE 1. Comparison of results obtained by EIAs and IDCF tests performed on 409 specimens from humans with coccidioidomycosis, noncoccidioidal pulmonary illness, heterologous fungal and nonfungal diseases, and no apparent illness

Patient condition (no. of specimens)	No. of positive specimens			
	IDCF	EIA	EIA for	
			IgG	IgM
Coccidioidomycosis (47)	47	47	43	36
Suspected noncoccidioidal pulmonary illness (345)	0	12	7	5
Aspergillosis (3)	0	0	0	0
Blastomycosis (5)	0	3	3	3
Histoplasmosis (3)	0	0	0	0
Human immunodeficiency virus (3)	0	0	0	0
No apparent illness (3)	0	0	0	0

ently healthy persons, are also shown in Table 1. With the exception of three of five blastomycosis case serum specimens and 12 of 345 specimens from patients with pulmonary infections other than coccidioidomycosis, none of the heterologous sera were positive. The IDCF test was totally specific and showed no reactivity with these specimens.

IgM-IDTP antibodies usually precede the development of IgG-CF antibodies, and they are usually associated with early primary infection. However, the IDTP-IgM test can be positive in some patients with chronic pulmonary cavities (3). Included among the 47 coccidioidomycosis case serum specimens were 14 known IDTP-positive specimens from patients with early disease. The EIA for IgG and IgM antibodies detected infection in all of these case specimens. However, when only an IgG or an IgM test was performed, antibodies were detected in only 13 or 12 of the 14 early case specimens, respectively. Interestingly, two of the IDTP-positive serum specimens were also negative by the EIA for IgM. Subsequent IDCF tests with these 14 specimens revealed that they were all positive.

Eighteen of the 47 proven coccidioidomycosis case specimens were examined by the CF test. Each of the 18 specimens reacted positively, with titers ranging from 1:2 to 1:512. All 18 were positive by the EIA, showing reactivity with IgG antibodies ($n = 5$), IgM antibodies ($n = 1$), or both types of antibodies ($n = 12$).

DISCUSSION

The EIA proved to be sensitive enough to detect antibodies in all the early and late coccidioidomycosis case specimens studied, regardless of whether they were reactive with the standardized IDCF (Table 1) or the IDTP or CF tests. As recommended by Meridian Diagnostics, Inc., both IgG and IgM tests must be performed to achieve this high level of sensitivity. Use of only the IgG test allowed detection of antibodies in 92% of the 47 coccidioidomycosis case specimens, whereas use of the IgM test alone allowed detection of antibodies in only 77% of the case specimens (Table 1).

Antibody was detected by the EIA in all 14 of the early case IDTP-positive serum specimens studied. Contrary to expectations, when the EIA for IgM was used alone, two (14%) of the early cases were missed. All 14 early case serum specimens were IDCF positive. However, none were positive by the CF procedure, thus indicating that the IDCF test is more sensitive than the CF test.

In a preliminary evaluation, Talbot et al. (4) reported the overall sensitivity and specificity of the EIA to be 96.5 and 84.3%, respectively. More recently, Wieden et al. (5) in studies with multiple serum samples from 24 patients with coccidioidomycosis found the EIA to be more sensitive (80%) than the standard ID tests and totally specific. Our data indicate that the EIA is totally sensitive (100% of the coccidioidomycosis case specimens were positive by the EIA) but lacking in specificity (96%). Accordingly, we are in agreement with the manufacturer of the EIA kit that a positive EIA should be confirmed by ID assays. However, the manufacturer indicated that the ID procedures are relatively insensitive and that an ID-negative reaction does not preclude the possibility of coccidioidomycosis (Premier Coccidioides EIA Package Insert; Meridian Diagnostics, Inc.). Our findings suggest that the IDCF and IDTP tests are as sensitive as and more specific than the EIA. The EIAs with either the IgG reagent or the IgM reagent showed nonspecific reactivity with sera from patients with blastomycosis and noncoccidioidal pulmonary disease. In contrast, the IDCF test demonstrated total specificity. The EIA can be used as an effective screening tool. It is not a quantitative assay, and thus it cannot be used to provide prognostic data as is the CF test (1, 3). Further improvements of the EIA are required either through antigen purification or quantitation of antibody to avoid false-positive reactions or cross-reactions, to obtain prognostic data, and to gain greater acceptance for and confidence in this procedure clinically.

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