JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1984, p. 530–532
0095-1137/84/090530-03$02.00/0

Vol. 20, No. 3

Evaluation of Five Commercially Available Immunodiffusion Kits for Detection of *Coccidioides immitis* and *Histoplasma capsulatum* Antibodies

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Received 9 January 1984/Accepted 16 May 1984

Five commercial test kits for the serodiagnosis of coccidioidomycosis and histoplasmosis based upon immunodiffusion were evaluated. The correlation of results with the test kits in the Clinical Laboratory varied from 71 to 100% for coccidioidomycosis. The correlation for coccidioidomycosis immunodiffusion testing varied from 57 to 83% when results from the test kits and the Mycology Research Laboratory were compared. Only 81% correlation was noted between the two laboratories when the same reference system was used. Results with the test kits for *Histoplasma* serodiagnosis and results from the Mycology Research Laboratory showed a correlation of 52 to 75%. There were no false-positive results with any system. All of the commercial kits were 100% specific for the diagnosis of both coccidioidomycosis and histoplasmosis, but the sensitivity of the immunodiffusion tests varied with the system used.

The complement fixation (CF) test has been the most widely used method for the serodiagnosis of mycotic infections, including coccidioidomycosis and histoplasmosis (5, 9). However, the complexity of the CF test has limited its performance primarily to reference laboratories and large hospital laboratories. In recent years, immunodiffusion (ID) testing has been suggested as an alternative means of serodiagnosis for mycotic infections, either alone or in conjunction with CF testing (6, 7). In 1963, Huppert and Bailey introduced an ID test for coccidioidomycosis which correlated qualitatively with the CF test (2), and they and Chitjian subsequently modified the method to make it readily adaptable for use in clinical laboratories (3, 4). *Histoplasma* ID testing with known reference antigens has also been shown to agree qualitatively with CF testing in most instances (6).

Because of its simplicity, reliability, and potential cost effectiveness, ID testing has become increasingly popular among laboratories. Thus, ID kits for fungal serodiagnosis have become commercially available from a number of manufacturers. Other than one report in which the Meridian Diagnostics kit was compared with latex agglutination tests and counterimmunoelectrophoresis systems for serodiagnosis of coccidioidomycosis and histoplasmosis (8), we are not aware of any previously published reports on the accuracy of commercially available ID systems. We decided to compare the qualitative results obtained with five different commercially available ID systems against results obtained with reference ID systems for both coccidioidomycosis and histoplasmosis. In addition, interlaboratory reproducibility was determined by performing ID testing with the ID-CF reference system for coccidioidomycosis in two different laboratories.

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MATERIALS AND METHODS

ID assay. Sera from 144 patients were sent to the Veterans Administration Mycology Research Laboratory and preserved by the addition of Merthiolate, refrigerated, and tested within 1 week. A reference ID system for *Coccidioides immitis* consisted of a standardized antigen (91F) and known human antisera (pool 23) which yielded a specific F precipitin band. The reference ID system for *Histoplasma capsulatum* antibodies consisted of reagents obtained from the Centers for Disease Control and was designed to give both H and M precipitin bands. The method of Huppert and Bailey (1, 3) was used for ID tests in both the Veterans Administration Mycology Research Laboratory and the Veterans Administration Clinical Laboratory. A 1.5% purified agar suspension in Sorenson phosphate buffer, to which 10 ml of a 1:1,000 dilution of Merthiolate was added, was prepared and dispensed into small plastic dishes (Rodac plates). These plates were refrigerated for a minimum of 2 h before use. A seven-well pattern cut into each agar plate consisted of a central well and six equally spaced peripheral wells. The peripheral wells were filled with known antisera and test sera. After a 1-h prediffusion period, the central well was filled with the control antigen. The plates were then incubated at room temperature (22°C) in a moist chamber and observed daily for 3 days for the development of a precipitin band which demonstrated a line of identity with the control system. ID tests with five different systems purchased commercially were performed according to the directions of each manufacturer. Positive results were determined in a similar manner. A single lot number of ID plates, *Coccidioides* antigen and antisera, and *Histoplasma* antigen and antisera were received from the following sources: Immuno-Mycologics, Inc.; Meridian Diagnostics, Inc.; M. A. Bioproducts; Nolan Biological Labs, Inc.; and American Scientific Products.

Data analysis. One hundred forty-four sera, consisting of 94 known to be positive for coccidioidomycosis, 36 known to

be positive for histoplasmosis, and 14 negative for both agents, were coded by the Mycology Research Laboratory and sent to the Clinical Laboratory for evaluation. After the results of all ID testing were completed, the code was broken, and the results were compared.

RESULTS

Table 1 contains the ID results for *C. immitis* antibodies obtained when the reference laboratories used the same reference system, as well as the results obtained with the five commercial kits with their own antigen-antibody systems. After 72 h, there was 80.9% agreement between the two laboratories on the reference ID test system. Correlation between the Mycology Research Laboratory results and the five commercial kits varied from 57.4 to 83.0%, whereas correlation between the Clinical Laboratory reference ID results and the five commercial kits varied from 71 to 100%. The ID results obtained with kits from M. A. Bioproducts and American Scientific Products were identical. Subsequently, it was learned that at the time of this study both companies were distributing fungal ID kits manufactured by Immuno-Mycologics, Inc., Lexington, Ky. (At the time of writing this paper, M. A. Bioproducts is distributing Meridian Diagnostics, Inc., reagents.)

The results of testing 36 positive histoplasmosis sera in the Mycology Research Laboratory and the Clinical Laboratory with five commercial kits are shown in Table 2. Correlation of results varied from 52.8 to 75%. All of the sera found to be positive by the Mycology Research Laboratory were also positive by CF testing, with the exception of two sera which were anticomplementary. There were no false-positive ID results in any system tested for either *Coccidioides* or *Histoplasma* antibodies.

DISCUSSION

ID results obtained with the commercial kits for demonstration of *C. immitis* antibodies, compared with the results of the Mycology Research Laboratory, showed a correlation of 57.4 to 83%. The correlation was much lower than the 71 to 100% agreement noted when ID results were compared with those of the Clinical Laboratory. In either laboratory, Meridian Diagnostics, Inc., had the highest correlation (83 and 100%, respectively) when compared with the ID-CF reference system. Since all of the kits were evaluated in the Clinical Laboratory by the same individual who interpreted the reference system tests, it was felt that comparison with the results of the Clinical Laboratory (71 to 100% correlation) was a truer reflection of the value of the commercial kits. The only ID results obtained in the Mycology Research Laboratory were the original reference tests.

TABLE 1. Comparison of ID results from five commercial systems and a reference system performed in two laboratories for *C. immitis* antibodies

System[a]	No. of positive tests recorded at (h):			Total positive results	% Correlation with:	
	24	48	72		MRL	CL
MRL				94[b]	100	
CL	76			76	80.9	100
IM	58	1		59	62.8	77.5
MER	78			78	83.0	100
MA	53		1	54	57.4	71.0
N	57	2		59	62.8	77.5
SP	52		2	54	57.4	71.0

[a] MRL, Mycology Research Laboratory; CL, Clinical Laboratory; IM, Immuno-Mycologics, Inc.; MER, Meridian Diagnostics, Inc.; MA, M. A. Bioproducts; N, Nolan Biological Labs, Inc., SP, American Scientific Products.
[b] Of the sera tested, >90% were positive at 24 h.

TABLE 2. Comparison of ID results from five commercial systems and a reference system for *H. capsulatum* antibodies

System[a]	No. of positive tests recorded at (h):			Total positive results	% Correlation with MRL
	24	48	72		
MRL				36[b]	100
IM	22	3		25	69.4
MER	24	1		25	69.4
MA	13		7	20	55.6
N	14	8	5	27	75.0
SP	13	4	2	19	52.8

[a] See Table 1, footnote *a*, for definitions of abbreviations.
[b] Of the sera tested, >90% were positive at 24 h.

Although the criteria for a positive result had been discussed before the study, there was poor overall correlation between the two laboratories (80.9%) with the ID-CF reference system. This led us to reexamine our methodologies. It was noted that the individual in the Clinical Laboratory interpreted a positive result as a definite bend in the control precipitin band between the reference well and test serum wells. A positive result in the Mycology Research Laboratory was interpreted as any deflection in the control precipitin band toward the test serum wells. The stricter criteria used in the Mycology Research Laboratory were a result of trying to determine the endpoint in quantitative ID studies. Using their criteria, we evaluated another 125 sera in the Clinical Laboratory. Ninety-seven percent (121 of 125) of the tests agreed, and there were two false-positive and two false-negative results compared with the results of the Mycology Research Laboratory. This interlaboratory variation points out the need for careful and consistent interpretation of the ID-CF test with strict criteria for the determination of a positive result. Wood et al. (10) also emphasized the necessity for careful interpretation of the endpoint when quantitative ID tests for coccidioidal antibody are performed.

ID results with the commercial kits for the demonstration of *H. capsulatum* antibodies showed a correlation of 52.8 to 75% when compared with results obtained in the Mycology Research Laboratory. The results demonstrated a lower correlation than expected because the ID tests were interpreted by the Clinical Laboratory before the difference in interpretation by the two reference laboratories, as discussed above, was noticed. One could assume from the comparative ID-CF data that the correlation for each system would be increased ca. 15% by using the stricter criteria of the Mycology Research Laboratory for interpretation of a positive result.

Land et al. (8) showed that ID testing for demonstration of *H. capsulatum* antibodies with a mycelial antigen was more sensitive than testing with a yeast phase antigen or a combined yeast and mycelial antigen. In all of the commercial systems evaluated in this study, a mycelial-phase antigen designed to yield both H and M precipitin bands was used. Although the control sera in the kits demonstrated both bands, test sera rarely showed evidence of the H precipitin band. Since this finding was common to all of the commercial kits, it was most likely a reflection of the sera tested and not a deficiency in the reagents used.

The five commercial systems evaluated recommended interpreting the *Coccidioides* and *Histoplasma* ID tests only at 24 h. Our practice is to examine them at 24, 48, and 72 h.

Little difference in results was noted for *Coccidioides* ID tests, but a considerable difference occurred with three of the kits with *Histoplasma* ID tests when only 24-h results were reported (Table 2). This difference suggests that increasing the incubation time to 72 h may increase the sensitivity of ID testing.

In all of the kits except one, an agar slide chamber was used. In the Nolan kit, a small petri dish containing agar is used and requires larger volumes of antigen and sera. The larger volumes may have been partially responsible for the increased number of *Histoplasma*-positive sera observed with this system. In addition, the increased distance between wells may have been responsible for the delayed positive results, as the diffusion time to reach optimal proportions may have been increased. It is also possible that the larger well pattern made the precipitin bands easier to visualize than did the small patterns incorporated on the agar slides used with the other kits. However, two other kits also demonstrated positive results at 72 h.

The overall results of this study indicate that the sensitivity of the ID tests varied with the commercial system used and with the antibody being tested for. If commercial ID tests are to be used in a given laboratory, we caution that the system should be tested against a reference system before final adoption.

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