

Comparison of Macrocomplement and Microcomplement Fixation Techniques Used in Fungus Serology

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A comparative evaluation was performed on the micro- and macrocomplement fixation (CF) tests that are used as standard procedures in the serodiagnosis of blastomycosis, coccidioidomycosis, and histoplasmosis. Tests with 937 sera from suspected and culturally proven cases of these diseases against yeast-form antigens of *Blastomyces dermatitidis* and *Histoplasma capsulatum* and against the soluble antigens, coccidioidin and histoplasmin, revealed that the microtiters were within ± 1 dilution of the macrotiters in 83 to 93% of the sera. Tests on randomly coded quality control sera revealed the microform of the CF test to be highly reproducible. Our studies indicate that the results obtained by the two tests have similar diagnostic and prognostic interpretations. Because of the sensitivity, reproducibility, economy, and ease of performance, the microtest is highly recommended for use in fungus serology.

The complement fixation (CF) test is the most widely used serological procedure in medical mycology. Properly performed, it can yield information of diagnostic and prognostic value. In the Fungus Immunology Unit of the Center for Disease Control, a standardized Laboratory Branch Complement Fixation (LBCF) test (5) is used to obtain presumptive evidence for a diagnosis of blastomycosis, coccidioidomycosis, or histoplasmosis (4). To accomplish this, each serum from a patient suspected of having a systemic mycotic infection is titrated against each of four optimally diluted antigens (2). For blastomycosis, the antigens are ground, yeast-form *Blastomyces dermatitidis* cells; for coccidioidomycosis, coccidioidin, and for histoplasmosis, two antigens are yeast-form cells of *Histoplasma capsulatum* and histoplasmin.

From 1962 to 1965, the tube or macro-LBCF test was performed in our laboratory. In this macrotest, a titer of 1:8 with either of the two *H. capsulatum* antigens is considered presumptive evidence of infection. Titers of 1:32 or greater with the *B. dermatitidis* and *H. capsulatum* antigens are highly suggestive of infection by these fungi and are of more diagnostic significance than the lower titers. Such titers with coccidioidin indicate active coccidioidal infections. Titer changes, of course, are invaluable in making a diagnosis, particularly with the *B. dermatitidis* and *H. capsulatum* antigens which demonstrate

greater cross-reactivity than coccidioidin. Four-fold changes in titer in either direction are considered significant indicators of disease progression or regression.

In 1965, growth of our serological services prompted us to evaluate the microadaptation of the LBCF test (5). Its potential advantages would be the savings resulting from use of smaller volumes of reagents and disposable microplates and the ease and speed of titrating two serum specimens against four antigens on each plate. The goal of the present study was to determine whether the results obtained by the macro- and microprocedures were comparable and to ascertain the within-run and day-to-day reproducibility of the microtest with the four fungus antigens.

MATERIALS AND METHODS

Human sera. Nine hundred and thirty-seven sera from suspected and culturally proven cases of blastomycosis, coccidioidomycosis, and histoplasmosis were coded and tested in groups by the macro- and micro-CF tests. The sera were not selected but were tested as they were received by the diagnostic laboratory; consequently, a large number of negative specimens was encountered.

Antigens. Four antigens were used in each test: a suspension of merthiolated, intact yeast-form cells of *H. capsulatum*; a soluble mycelial filtrate antigen, histoplasmin; a suspension of ground, yeast-form *B. dermatitidis* cells; and a soluble, mycelial filtrate antigen of *Coccidioides immitis*, coccidioidin (2).

CF tests. Sera were titrated by the LBCF test and the micromodification (5). These tests utilize five 50% units of complement. The antigen-antibody-complement mixture is incubated for 15 to 18 hr at 4 C. Sera demonstrating 30% hemolysis or less at a particular dilution were considered positive. The initial 1:8 dilutions of heat-inactivated sera were prepared by using conventional pipettes and transferred to the microplates. Ensuing dilutions were made with micro-loops.

Quality control study. Randomly coded quality control sera from 6 pools were included in 42 routine diagnostic runs covering a period of 7 months. The titers for these pools ranged from 1:8 to 1:2,048. Each of the 42 runs included five blind control specimens. One or two pairs of duplicates were represented in the five controls for a given run. After 42 runs, 64 pairs of titers were available on which to base an estimate of within-run reproducibility. The distribution of titers for each pool over the 42 runs also provided the basis for estimating day-to-day reproducibility (1).

RESULTS

Tables 1 to 4 show the distribution of macro- and microtiters in tests with each of the fungus antigens against the 937 sera. Each table is bordered by geometric means indicating the average microtiter obtained for all the sera with a given macro-titer and vice versa.

From the data, it is apparent that there is an overall correlation between macro- and micro-titers. The "scatter" of microtiters in most instances is within the expected range of reproducibility (± 1 dilution) for the test. In general, macrotiters tend to be somewhat higher than microtiters, particularly with high titered sera. However, 2 to 8% of the sera that were macro-negative had low positive microtiters. These percentages were significantly greater than the percentages (0.3 to 2.4%) of sera that were micro-negative and macro-positive.

TABLE 1. *Distribution of complement fixation titers with histoplasmin antigen among 937 sera*

Macro-titers	Microtiters						Total	GM ^a
	<8	8	16	32	64	128		
<8	812	36	4	1			853	<8
8	1	25	6				32	9
16	3	2	10				15	11
32	1		6	10	4		21	27
64				3	8	1	12	57
128					2	1	3	81
Total	818 ^b	63	26	14	14	2	937 ^b	
GM	<8	<8	13	32	58	91		

^a Geometric mean.

^b Includes one serum with a macro-titer of 1:1,024.

TABLE 2. *Distribution of complement fixation titers with yeast-form Histoplasma capsulatum antigen among 937 sera*

Macro-titers	Microtiters							Total	GM ^a
	<8	8	16	32	64	128	256		
<8	763	28	6	2		2		801	<8
8	7	29	10					46	8
16	8	10	13	2				33	10
32	3	2	7	7	4	2		25	23
64	1		2	5	12	1		21	43
128				1	3	4		8	83
256						2		2	128
Total	783 ^b	69	38	17	19	11		937 ^b	
GM	<8	<8	13	31	62	57			

^a Geometric mean.

^b Includes one serum with a macro-titer of 1:2,048.

TABLE 3. *Distribution of complement fixation titers obtained with coccidioidin antigen among 937 sera*

Macro-titers	Microtiters							Total	GM ^a
	<8	8	16	32	64	128	256		
<8	898	14	3					915	<8
8	1	1	2					4	10
16	1	2	6	1				10	13
32				1				1	32
64					3	1		4	76
128						2		2	128
256	1							1	
Total	901	17	11	2	3	3		937	
GM	<8	<8	10	23	64	102			

^a Geometric mean.

Table 5 summarizes the relationship of macro- and microtiters for each of the four tests. Only sera with at least one positive titer (macro or micro) are included in this table to avoid the predominance of double-negative sera in some tests. From the data shown, it is evident that the relationship of macro- and microtiters for the four tests is similar. Except for tests with the yeast-form antigens, microtiters were, on the average, significantly higher than macrotiters. This is due almost entirely to sera that had negative macrotiters and positive microtiters. With higher titered sera, the microtiters tend to be lower than the macrotiters. Microtiters were within ± 1 dilution of the macrotiters of 83 to 93% of the sera.

Table 6 summarizes the results on the blind control sera with the four fungus antigens. With the exception of the coccidioidin tests, the within-run reproducibility was 100%. Only 1.6% of the duplicate pairs of coccidioidin reactors yielded fourfold differences in titer; reproducibility, therefore, was 98.4%.

TABLE 4. Distribution of complement-fixation titers obtained with yeast-form *Blastomyces dermatitidis* antigen among 937 sera

Macrotiters	Microtiters								Total	GM ^a
	<8	8	16	32	64	128	256	512		
<8	738	49	14	3	1				805	<8
8	5	47	9		1				62	9
16	4	6	22	4	1				37	14
32		1	6	15					22	25
64				3	1				4	39
128						2	1		3	161
256	1					1	1		3	181
512						1			1	128
Total	748	103	51	25	4	4	2		937	
GM	<8	<8	11	24	14	215	181			

^a Geometric mean.

TABLE 5. Distribution of pair relationships between macro- and microcomplement-fixation titers with four antigens

Relationship of macro- and microtiters	Histoplasmin		<i>H. capsulatum</i> yeast-form		<i>B. dermatitidis</i> yeast-form		Coccidioidin	
	No.	%	No.	%	No.	%	No.	%
Macro 5 dilutions higher				1 (1)				
Macro 4 dilutions higher				3 (2)				
Macro 3 dilutions higher	1	(1)		13 (7)		6 (3)	1	(3)
Macro 2 dilutions higher	3	(2)		34 (20)		21 (10)	3	(8)
Macro 1 dilution higher	14	{(44)}		65 (37)		88 (44)	13	{(34)}
Macro equal to micro ^a	54	{(44)}		45 (26)		63 (32)	18	{(47)}
Macro 1 dilution lower	47	{(38)}		8 (5)		15 (8)	3	(8)
Macro 2 dilutions lower	4	(3)		2 (1)		4 (2)		
Macro 3 dilutions lower	1	(1)		2 (1)		1 (1)		
Macro 4 dilutions lower				2 (1)				
Macro 5 dilutions lower								
Total	124	(100)	173	(100)	198	(100)	38	(100)
Avg difference ^b		-0.3		0		-0.4		-0.5
Percentage agreement ^c		93		83		86		89

^a Sera with both titers negative were excluded to avoid their predominance on the results.

^b Average of macro- minus microtiters in terms of number of dilutions.

^c Per cent of sera with macro- and microtiters within ± 1 dilution of the average difference. Includes sera enclosed by brackets.

TABLE 6. Routine within-run reproducibility of the microtest with four antigens

Agreement of duplicate titers	Histoplasmin		<i>H. capsulatum</i>		<i>B. dermatitidis</i>		Coccidioidin	
	No.	%	No.	%	No.	%	No.	%
Same.....	53	82.8	52	81.2	55	85.9	56	87.5
One dilution apart.....	11	17.2	12	18.8	9	14.1	7	10.9
Two dilutions apart.....							1	1.6
Total no. of duplicates.....	64	100.0	64	100.0	64	100.0	64	100.0

Day-to-day reproducibility with the histoplasmin, *H. capsulatum* yeast-form, *B. dermatitidis* yeast-form, and coccidioidin antigens was 97.0, 95.5, 91.8, and 97.0%, respectively.

DISCUSSION

The LBCF test in both the macro- and micro-form is used by many public health laboratories for the serodiagnosis of blastomycosis, coccidioidomycosis, and histoplasmosis. Many efforts are being made to have these procedures adopted as standard CF tests. Success would lead to the use of a uniform diagnostic procedure for the mycoses throughout the world. However, before such universal use could be advocated, the micro-procedure had to be compared with the clinically evaluated tube LBCF test (3). If there were significant variations between the two tests, the relationship of microtiters to clinical disease would have to be separately determined.

Our studies indicate that results obtained by the microtest are comparable to those obtained by the macroprocedure. With the four fungus antigens, 83 to 93% of the microtiters were within one dilution of the macrotiters. In general, with low serum dilutions (1:8 and 1:16), the microtiters were, on the average, higher than the macrotiters. This result appeared to have been due primarily to sera that were negative in the macrotest and positive in the microtest. On the

other hand, with the high titered sera, macrotiters were slightly higher than microtiters. However, the number of these discrepancies was insignificant, and, from a practical point of view, the results obtained by the micro- and macro-LBCF tests were similar. Consequently, the diagnostic and prognostic interpretations of these tests are similar. The micro-LBCF test was found to have a high level of reproducibility seldom achieved in many other routine serological tests. Because of the demonstrated ease, economy, accuracy, sensitivity, and reproducibility of the micro-LBCF test, we urge that all laboratories consider adopting this procedure. Reagents for these tests are available from various sources.

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