Serology of Systemic Fungus Diseases

LEO KAUFMAN, Ph.D.

SEROLOGIC procedures were first applied to the diagnosis of mycotic diseases in the early 1900's (*1*, *2*). Since then new methods for serodiagnosis have been continuously developed and existing ones refined. The most significant changes have been in the laboratory procedures for diagnosis of infections caused by *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, and *Cryptococcus neoformans*. Definite trends in diagnosis of mycotic infections during the last few years have included adoption by more and more public and private laboratories of serologic procedures, replacement of conventional tube tests with micro methods, greater use of immunodiffusion techniques, a quest for more specific antigens, and increased efforts to standardize test procedures and reagents.

I intend to review briefly the diagnostic significance of these trends and to comment on research being directed toward improvement of current methodology in the diagnosis of the important systemic mycotic diseases—histoplasmosis, coccidiodomycosis, North American blastomycosis, and cryptococcosis.

Histoplasmosis

The tests most widely used currently for detection of *H. capsulatum* antibodies are the complement-fixation and agar gel precipitin tests. The soluble mycelial antigen, histoplasmin, is used in both tests, but whole yeast-form cells are also used as a second antigen in the complement-fixation test. Use of both these antigens provides maximal diagnostic coverage since serums from culturally confirmed cases of histoplasmosis may react to only one of these antigens (*3*, *4*).

A complement-fixation titer of 1:8 or greater with either the histoplasmin or the yeast-phase antigen, is generally considered presumptive evidence of histoplasmosis. Table 1 shows typical immunological reactions observed with serums from persons with culturally proven histoplasmosis. Apparently patients suffering from histoplasmosis may be immunologically responsive to all the antigens, to some of them, or to none. It should be emphasized, however, that a lack of immunological response does not exclude histoplasmosis, particularly when only a single specimen has been tested and when the clinical picture strongly suggests pulmonary mycotic disease. Conversely, positive histoplasma titers do not always indicate active histoplasmosis. Typical positive serologic reactions have been recorded in the Fungus Serology Laboratory, Communicable Disease Center, which did not correlate with the attending physicians' diagnoses after the physicians had examined pertinent clinical, histopathological, serologic, and laboratory data (table 1). Whether these results represent double infections, incorrect diagnoses, or nonspecific reactions is not clear.

Observations such as these have been adequately documented in the literature by Schubert and Wiggins (*5*). Along similar lines, Mays and co-workers (*6*) reported that, in 1,024 cases of tuberculosis studied, 4 percent of the patients had positive serologic reactions to fungal antigens. Titers ranging from 1:8 to greater than 1:32 were observed in 47 cases

Dr. Kaufman is in charge of the Fungus Serology Laboratory in the Mycology Unit, Mycology and Parasitology Section, Communicable Disease Center, Public Health Service, Atlanta, Ga. *This paper is based on a report presented by Dr. Kaufman at a round table on "Medical Mycological Topics of Broad Interest" at the annual meeting of the American Society for Microbiology in Atlantic City, N.J., May 1965.*

diagnosed as tuberculosis, of which 79 percent were bacteriologically proven. It is also evident that, although high titers of 1:32 or greater may be of more diagnostic significance than lower titers, they cannot be relied upon as the sole means of diagnosis. Since common antigens are shared among fungi (*7*, *8*) and between fungi and other micro-organisms (*9*), serologic reactions to the crude *H. capsulatum* antigens currently in use must be prudently interpreted.

The histoplasmin skin test has been an epidemiologic tool and, on a limited basis, a diagnostic aid, for many years. Results of many recent investigations, however, indicate that levels of complement-fixing antibodies to *H. capsulatum* antigens may be significantly increased after a single histoplasmin skin test (*10–12*).

In 1961, Nicholas and associates (*11*) observed that, in 4 of 23 histoplasmin-sensitive persons with serologically negative reactions, complement-fixation titers of 1:8 and 1:16 were evoked 2 weeks after administration of a single histoplasmin skin test. Later, Sigrest and coworkers (*13*) observed a similar reaction in 1 of 14 hypersensitive patients. In this particular patient, a blood serum specimen 2 weeks after the skin test was negative, but a titer of 1:8 was noted in a serum specimen taken 4 weeks after the test. Recently, Campbell and Hill (*12*) reported that conversions in complement-fixation titers from negative to a range of 1:8 to 1:256 were produced in 7 of 12 histoplasmin-sensitive persons 5 to 30 days following a single histoplasmin skin test.

The apparent influence of a single histoplasmin skin test on complement-fixation titers appears to cast doubt on the reliability of the histoplasmin complement-fixation test. Additional studies, however, are needed to investigate the validity of these observations. In the meantime the histoplasmin skin test should be used with discrimination, or not at all, on persons who are subsequently to be serologically tested.

Many workers have considered the agar gel test to be potentially useful in differentiating an antibody response due to a skin test from an antibody reaction due to infection. Diagnostically important precipitin bands were first described by Heiner in 1958. He designated as the "M" band a band that was the result of a reaction between the histoplasmin antigen and the antibody produced in response to skin testing. A band that resulted from reaction with the antibody and was uninfluenced by skin testing but was compatible with current infection, he termed the "H" band. Although the H band is always associated with the M band, the converse is not known to be true. Yet the M band has been considered as presumptive evidence of infection by *H. capsulatum* (*15*). Recent

Table 1. Complement-fixation and skin test results in patients with culturally proven histoplasmosis and in patients with other diseases

Diagnosis and case number	Complement-fixation titer [1] by antigen			Skin test reaction [2]
	Histoplasmin	*Histoplasma capsulatum* (yeast-form)	*Blastomyces dermatitidis* (yeast-form)	
Histoplasmosis:				
1	64	256	256	—
2	64	64	64	+
3	64	256	256	—
4	512	128	16	+
5 [3]	8	64	16	+
6	8	0	0	+
7	32	0	0	+
8	0	8	0	+
9	0	0	0	—
10	0	0	0	+
11	0	8	0	+
12	0	16	0	+
13	0	32	0	+
14	0	32	32	+
15	0	0	8	+
16	0	0	32	—
Other diseases:				
1 Tuberculosis	16	16	0	+
2 Pneumonia	16	128	0	+
3 Tuberculosis	128	32	16	+
4 Uveitis	8	32	0	+
5 Cryptococcosis	0	8	64	+
6 Tuberculosis	0	64	0	—
7 North American blastomycosis	64	64	0	+
8 Cancer	0	16	0	—
9 Toxoplasmosis	0	8	0	—

[1] Reciprocal of dilution.
[2] Skin test with histoplasmin.
[3] Although all 25 patients were tested with the antigen coccidioidin, only one patient (histoplasmosis case number 5) responded positively—with a titer of 1:8.

studies (*4*) have shown that results obtained with the agar gel technique correlate well (82 percent) with those obtained with the complement-fixation test when histoplasmin antigens but not yeast-phase cells of *H. capsulatum* are used (table 2). If the M band appears only in response to skin-test stimulation, the agar gel test would serve to differentiate persons responding only to a histoplasmin skin test from those responding to the infectious agent. My experience, as well as that of Wiggins and Schubert (*16*), has been, however, that serums of many patients with culturally proven histoplasmosis apparently only contain antibody to the M antigen. Thus, interpretation of an M band solely as a response to a skin test would lead to diagnostic errors and seriously limit the usefulness of the agar gel test. In the absence of a recent skin test the M factor actually may serve as an early indicator of disease, appearing before the H factor and disappearing more slowly.

Perhaps the greatest limitation of serologic procedures for diagnosis of histoplasmosis lies in the sensitive yeast-form antigens, since reactions to these antigens are not generically specific. Labzoffsky and co-workers (*17*) reported the extraction of specific antigens from *H. capsulatum*. To date, however, workers in our laboratory, using either the techniques described by Labzoffsky or other procedures, have not succeeded in preparing antigens that are more stable or more specific than the crude preparations currently used.

Coccidioidomycosis

Of all the serologic tests currently applied to the systemic mycoses, perhaps the most thoroughly studied and useful are those for diagnosis of coccidioidomycosis. Comprehensive studies have shown that complement-fixation and precipitin tests are invaluable aids in the diagnosis and prognosis of this disease. Both test systems incorporate the soluble antigen coccidioidin, a pool of culture filtrates derived from several strains of *C. immitis*. The specificity of these tests was established by Smith and associates (*18*) when they demonstrated no cross-reactions with serums from patients with viral, rickettsial, bacterial, and most other mycotic infections. These workers noted, however, that cross-reactions might occur with histoplasmosis and North American blastomycosis. They emphasized the relative importance of both tests in the diagnosis of coccidioidomycosis. Of 3,219 patients with uncomplicated primary coccidioidomycosis, 78 percent reacted to the precipitin test, and 56 percent reacted to the complement-fixation test. A combination of the two tests made it possible to detect more than 90 percent of the primary infections in which symptoms were present. These data signify that maximum diagnostic coverage for coccidioidomycosis can be attained only when both tests are used. Smith and associates emphasized that, while in many early cases the patients' blood serums fix the complement and contain demonstrable precipitins, many serums containing precipitins fail to fix the complement. They recognized the complement-fixation test as the most effective in determining disseminated disease and the precipitin test as most effective in picking up early infections.

Contrary to Smith's observations, which were based on the 100 percent end-point test, Schubert and Hampson (*19*), who used 50 percent and 100 percent complement-fixation tests, reported that the complement-fixation test, in addition to making it possible to detect *C. immitis* antibody in serums from patients with disseminated disease, more effectively detected *C. im-*

Table 2. Comparison of results of the complement-fixation and agar gel tests in patients with culturally proven histoplasmosis

Case number	Complement fixation titer [1] with—		Agar gel reactions
	Histoplasmin antigen	*H. capsulatum* (yeast-form antigen)	
1	32	128	+
2	16	16	+
3	32	128	+
4	8	0	+
5	8	0	+
6	0	32	+
7	0	16	−
8	0	8	−

[1] Reciprocal of dilution.

mitis antibody in primary cases than did the tube precipitin test. Their study requires confirmation since it was limited by the number of serums available from patients with well-defined early cases. Of five serums from patients with disseminated cases taken in the first month of disease, all five were positive by the complement-fixation test, whereas only two were positive by the precipitin test. These workers also reported that the agar gel method as they performed it, although more sensitive than the tube precipitin test, could not serve as an inclusive screening procedure since it appeared to be less sensitive than the complement-fixation test.

The value of low complement-fixation titers in the diagnosis of coccidioidomycosis is continually debated. Smith and co-workers (*18*) stress the importance of titers of 1:2 and 1:4 in primary nondisseminated infections. Of 1,789 primary cases in which the patients' serums showed positive complement-fixation titers, 716 had maximal titers of 1:2, and only 43 had titers greater than 1:4. Titers of 1:2 and 1:4 have been found to be indicative of early, residual, and meningeal coccidioidomycosis. Other investigators (*19*, *20*) have essentially substantiated the value of the 1:2 and 1:4 titers as indicators of disease but have also encountered such titers in serums from patients known not to have coccidioidomycosis. Thus, when such low titers are obtained, a diagnosis of coccidioidomycosis must be based on repeated serologic tests and preferably, on clinical and mycological studies.

Negative serologic results do not exclude coccidioidomycosis. Table 3 shows the complement-fixation titers obtained with serums from patients with proven cases of coccidioidomycosis. Two of eight patients had negative coccidioidin serologic reactions.

The coccidioidin skin test is considered a valuable screen for serologic testing. Smith and co-workers (*18*) never observed positive serologic results in patients with primary coccidioidomycosis infection or with impending dissemination in the absence of a positive skin test. Unlike the serologic reactions noted after administration of the histoplasmin skin test, coccidioidin skin testing does not appear to elicit humoral antibodies.

Currently a number of laboratories are evaluating or are using serologic procedures for coccidioidomycosis that are simpler to perform, yield results more rapidly, and are less expensive than the conventional tube complement-fixation tests. Experience in these laboratories indicates that results obtained by these new procedures are comparable to results by conventional methods. Two such techniques which are becoming widely employed are the immunodiffusion and micro complement-fixation tests.

Recently Huppert and Bailey (*21*) prepared two types of antigen solutions to use in an immunodiffusion test for coccidioidomycosis. One was prepared from the culture filtrates of *C. immitis* and purified and concentrated by ultrafiltration (10X). It gave results similar to those obtained with the complement-fixation test antigen. The second solution was an immunodiffusion antigen prepared from a toluene lysate of a fungus mat which had been heated at 60° C. for 30 minutes to inactivate complement-fixing antigens. This antigen usually produces a single line in the agar gel test. This

Table 3. Complement-fixation test results [1] in patients with culturally proven coccidioidomycosis and North American blastomycosis

Diagnosis and case number	Histoplasmin	*H. capsulatum* (yeast-form)	*B. dermatitidis* (yeast-form)	Coccidioidin
Coccidioidomycosis:				
1	0	0	0	64
2	0	0	32	256
3	0	0	0	128
4	0	0	16	0
5	0	0	0	8
6	0	0	0	16
7	0	0	0	1, 024
8	0	0	0	0
North American blastomycosis:				
1	0	0	0	0
2	0	0	0	0
3	0	16	16	0
4	0	64	0	0
5	0	0	0	0
6	16	16	64	0
7	0	0	0	0
8	0	0	16	0
9	0	0	8	0

[1] Expressed as reciprocal of dilution.

band develops only with serums positive in the tube precipitin test.

Studies indicate that the immunodiffusion test is more sensitive than the tube test. According to Huppert and Bailey (*22*), immunodiffusion tests, which are easy to perform and yield results in 24 to 72 hours, can be used as qualitative screening tests. Positive reactions will identify, for the serologist, with an accuracy greater than 95 percent, those specimens which will be positive in the tube precipitin and complement-fixation tests.

A number of laboratories have adapted micro complement-fixation techniques (*23*) to fungus serology. These procedures, which employ a total volume of 0.125 ml. of reagents, enable a technician to titrate approximately 150 to 200 serum specimens in a half day. Laboratories using the micro complement-fixation test have reported obtaining reproducible results that show excellent correlation with results by the conventional tube tests. Aside from reduced labor costs with this test because of quick quantitation, a considerable savings accrues because fewer reagents and disposable plastic plates are used.

North American Blastomycosis

The serologic procedures for diagnosis of North American blastomycosis are the least reliable of the fungal serologic procedures currently in use. The ineffectiveness of these serologic tests has been attributed, for the most part, to the use of antigens that are either nonspecific, insensitive, or both.

A recent Veterans Administration cooperative study (*24*) showed that positive blastomycosis skin tests or positive complement-fixation tests were obtained in less than 50 percent of proven cases. Of 105 proven cases in which serums were tested by complement-fixation techniques using yeast-form or mycelial antigens, only 51 were positive. In another set of 136 proven cases, 56 patients showed positive skin-test reactions to such antigens. In the majority of cases the skin tests were made with blastomycin; in a few instances, however, tests were performed with soluble antigens derived from yeast-form cells of *B. dermatitidis*.

In an additional investigation by the VA group, skin tests and complement-fixation tests were performed on 86 patients with culturally proven cases of North American blastomycosis. In 20 percent (17 cases) both tests were positive; in 36 percent (31 cases) both tests failed to elicit an immunological response in the host.

Studies performed at the Fungus Serology Laboratory, Communicable Disease Center, have also demonstrated the need for more sensitive and specific complement-fixation test antigens to detect blastomycosis. Of 21 serums from patients with proven cases of blastomycosis recently studied, only 8 reacted to the ground yeast-form antigens used. Typical results obtained in our laboratory are shown in table 3. Among the positive serums, only a small number reacted solely to the *B. dermatitidis* antigen; others reacted to both the homologous and *H. capsulatum* antigens or to either or both of the *H. capsulatum* antigens alone.

Our records show that the *B. dermatitidis* antigens readily react to serums from patients who show no evidence of North American blastomycosis. Complement-fixation titers have been recorded for serums from patients having histoplasmosis, coccidioidomycosis, mucormycosis, tuberculosis, sarcoidosis, and malignant diseases.

Recently, Abernathy and Heiner (*25*) attempted to overcome the limitations of the complement-fixation test with *B. dermatitidis* antigens by using the agar gel precipitin test and concentrated soluble antigens derived from the mycelial growth of *B. dermatitidis*. These investigators noted considerable antigenic variations among the three strains of the fungus used. Ion-exchange chromatography revealed that the number of antigens varied from four to five, depending upon the strain studied. Among 22 serums from persons with proven cases, 14 contained precipitins. To determine the correlation between results obtained by the complement-fixation tests and by the immunodiffusion tests, these workers simultaneously tested serums from 17 persons with proven cases of North American blastomycosis by the two procedures. The correlation was good; 13 of the 17 serums reacted positively by the two methods. Although cross-reactions occurred with serums from patients with coccidioidomycosis and histoplasmosis, the heterologous

reactions could be distinguished from specific reactions by demonstrating a common or "c" type precipitin or antibody in such serums, a factor lacking in the serums of persons infected with *B. dermatitidis*.

Possibly the precipitin test could aid in differentiating serums that specifically react to the *B. dermatitidis* complement-fixation antigens from those that react nonspecifically.

Dr. R. S. Abernathy of the University of Arkansas, Little Rock, believes that the precipitin test is more specific than the complement-fixation test (personal communication, March 9, 1965). Recent studies, however, show that it may be less sensitive. At the moment, Abernathy recommends both tests whenever blastomycosis is suspected.

Dr. J. F. Busey of the Veterans Administration Hospital, Jackson, Miss., has also obtained excellent results with the agar gel precipitin test and recommends its use in the diagnostic laboratory (personal communication, May 27, 1965). He uses soluble mycelial or yeast-form antigens of *B. dermatitidis*, or both. Of 45 serums from persons with proven cases, 43 contained precipitins. Serums from persons infected with *H. capsulatum* gave false-positive reactions. With few exceptions, however, Busey was able to differentiate such serums from the blastomycosis specimens by simultaneously running immunodiffusion tests with histoplasmin containing the H and M antigens of *H. capsulatum*.

Blastomycin and a yeast-form vaccine are used to demonstrate skin-test hypersensitivity to the etiological agent of North American blastomycosis. Nevertheless, recent studies have indicated that blastomycin is inadequate for this purpose because it is neither specific nor sensitive. In 1963, Dr. A. Balows of the University of Kentucky, Lexington (*26*), simultaneously skin-tested 25 persons with active blastomycosis, using blastomycin and a yeast-form vaccine prepared from *B. dermatitidis*. Only 1 of these persons had a positive skin test with blastomycin, whereas 21 of the 25 had positive skin tests with the yeast-form vaccine. Balows has thus shown that the two antigens do not elicit similar responses, and his findings support the view that hypersensitivity to current *B. dermatitidis* infection is best demonstrated by intradermal injection of a yeast vaccine rather than of blastomycin.

More recently, Balows skin-tested 27 persons who had had blastomycosis 6 months or more before administration of the antigen (personal communication, March 10, 1965). In addition, he skin-tested 75 persons who from all indications were free of *B. dermatitidis* infection. Three antigens were evaluated: a commercial and a noncommercial blastomycin and a heat-killed yeast-form vaccine. The blastomycin antigens were found to be virtually worthless in that they showed no significant degree of sensitivity. The yeast-cell suspension showed a higher degree of sensitivity than the soluble antigens in that it reacted in 35 to 38 percent of the serums of the persons who recently had had North American blastomycosis. No statistically significant cross-reactions occurred with either of the three skin-test antigens in serums of persons whose skin tests were positive to histoplasmin.

It is interesting to compare the number of persons with positive skin tests in the group of recovered blastomycosis patients with the number in the group of actively infected patients studied in 1963. Eighty-four percent of the actively infected persons reacted to the skin-test antigen in contrast to 35 to 38 percent of those who had recovered. It would appear that the yeast-form vaccine of *B. dermatitidis* is not as effective a screening or diagnostic tool as histoplasmin or coccidioidin. The most opportune time for detecting the hypersensitive state is apparently when the disease is most actively manifesting itself or when organisms are demonstrable in clinical materials.

The available data suggest that, if a reliable skin test antigen for North American blastomycosis is to be produced, it will probably be derived from the yeast form of *B. dermatitidis* rather than from the mycelial state.

The large percentage of negative reactions observed with all the serologic procedures developed for North American blastomycosis emphasizes the necessity for further research.

Cryptococcosis

Cryptococcosis is an important cosmopolitan disease of frequent occurrence. Development of a reliable serologic test for it would hasten

diagnosis and assist in determining the effect of chemotherapy. Although many serologic procedures for detecting cryptococcosis have been studied, few have been adequately evaluated and documented.

In 1951, Neill and co-workers (*27*), using the complement-fixation test, demonstrated the presence of cryptococcal antigens in the spinal fluid, blood, and urine of a person with cryptococcosis. Similarly, Anderson and Beech (*28*) in 1958, using cryptococcal polysaccharide, also showed significant titers present in the spinal fluid of two patients with meningitis.

In a 1958 report, Vogel and Padula (*29*) showed that the indirect fluorescent antibody technique could be used to detect cryptococcal antibody in a person having cryptococcosis. In a second report, Vogel and co-workers (*30*), using the indirect fluorescent antibody procedure, detected antibodies in six of seven serums from persons with culturally proven cases of cryptococcosis. They also noted positive reactions in 8 percent of the serums from persons without evidence of cryptococcosis (339 specimens). None of the titers recorded, however, equaled those obtained in serums of patients suffering from cryptococcosis. In the Fungus Serology Laboratory, we have used Vogel's indirect fluorescent antibody procedure to study serums from 23 persons with culturally proven cases of cryptococcosis. Eighteen of the specimens were positive by this procedure. None of six normal serums studied showed staining at a significant level.

More recently, Pollock and Ward (*31*) developed a hemagglutination test for cryptococcosis with crude *C. neoformans* type A capsular polysaccharide fixed to human type O red blood cells as antigen. They reported significant titers in serums from two patients with cryptococcosis. Cross-reactions in low titer only were noted in serum from a patient with disseminated histoplasmosis.

In 1963, Bloomfield and co-workers (*32*) demonstrated antigens of *C. neoformans* in the serum or cerebrospinal fluid, or both, of patients with cryptococcal meningitis by agglutinating antibody-coated latex particles. They detected antigen in five of seven cases; the titers of the initial serum specimens were 1:16 or 1:32. In six of eight proven cases, titers ranging from 1:2 to 1:2,048 were demonstrated in initial specimens of cerebrospinal fluid. The authors reported that this procedure appeared to have adequate specificity and prognostic, as well as diagnostic, value. More recently, Dr. Morris Gordon, associate research scientist, New York State Health Department, Albany, used the tube agglutination test to successfully detect *C. neoformans* antibodies in 6 of 14 culturally proven cases (personal communication, February 23, 1965).

Although most of the procedures discussed have not been extensively evaluated, they at least signify that *C. neoformans* stimulates antibody production in man. They also indicate that serologic tests are available for diagnosis and prognosis. On the basis of these promising studies it appears that hemagglutination, agglutination, and fluorescent antibody tests, or a combination of these techniques, may provide the mycologist with methods suitable for the detection of the cryptococcal antibody.

Comments

With the exception of the tests for North American blastomycosis and cryptococcosis, serologic methods for diagnosis of systemic fungus diseases have become so effective that many State and other public health laboratories have adopted them for routine use. Lack of conformity in test performance, however, makes even rough comparisons of serologic results difficult. Variations in the type of antigen used, the ratio of antibody to antigen, and the temperature and time allowed for interaction of the test components affect the degree of sensitivity of a test and hence the resulting titer.

Use of standard antigens and antiserums is important with reagents of diagnostic value. Unless well-defined standards are included in each test, various laboratories will obtain results that have little or no relationship to one another.

Studies conducted by Smith and co-workers reported in 1957 (*33*) and studies by the Communicable Disease Center, Laboratory Consultation and Development Section, in collaboration with the States in 1964 have shown the considerable variations in laboratory results when serums or antigens are distributed to various laboratories performing similar or different complement-fixation tests for fungal antibodies.

Some investigators found that maintenance of a uniform program was difficult. They have expressed the belief that variable results due to changes in methodology by laboratory personnel (use of homemade reagents and other innovations) will eventually sabotage and cause termination of a uniform program. The experience of others, however, namely, the Venereal Disease Research Laboratory of the Public Health Service, has been that with proper standards and supervision an evaluation program can operate continuously and yield reproducible results that are comparable from laboratory to laboratory.

Preliminary analysis of a recent survey conducted by the Laboratory Consultation and Development Section of the Communicable Disease Center demonstrated that, of 13 different public health laboratories in which the same complement-fixation test was performed with standard antigens and antiserums, more than 90 percent reported titers within a twofold range of the control laboratory. These results point out the potentialities of a rigidly controlled test and evaluation program.

The maximal value of serologic tests will be achieved only when laboratories use standard reagents and procedures. The ultimate goal of serologists in medical mycology is the development of specific and potent fractions of various fungus antigens. To date no such products exist. Choice of procedures may be difficult even though evaluations have already been performed on some of the tests currently used. The decision as to procedure will depend of course upon the choice of antigens, concentration of reagents used, and other factors previously mentioned. Once test procedures are selected, they must be strictly adhered to.

The type, purity, and source of the antigens and the control serums must also be determined. Adequate comparison of lots of antigens currently in use should be made and lots of antigens designated for future use compared with them. Through exchange of test serums, the work of various laboratories needs to be evaluated periodically. Only through use of such reference standards can meaningful comparisons be made.

The Communicable Disease Center recognizes the need for achieving uniformity in testing and has developed and promoted the use of a standardized complement-fixation procedure, useful with all diagnostic complement-fixation antigens (*34*). It makes available to State health departments control serums and antigens in order to establish uniformity in procedures and performance.

Summary

A wide variety of serologic tests are currently performed as indicators of infection caused by *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, and *Cryptococcus neoformans*. Their diagnostic usefulness varies. The need for more sensitive and specific fungal antigens for such tests has stimulated research, but work to date directed toward this goal has been disappointing.

Adoption of uniform tests procedures with standardized antigens and reference serums would permit the best use of serodiagnostic tests in mycology and the attainment of comparable and meaningful results by various laboratories.

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