Improved Version of the Exoantigen Test for Identification of *Coccidioides immitis* and *Histoplasma capsulatum* Cultures

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A rapid and simple method for extracting specific cell-free antigens of *Coccidioides immitis* and *Histoplasma capsulatum* from agar slant cultures was developed. The extracts were analyzed in immunodiffusion tests for the presence of *C. immitis* or *H. capsulatum* specific antigens. These extracts were compared with culture filtrates of brain heart infusion broth subcultures in tests with 32 isolates of *C. immitis* and *H. capsulatum* and 13 other fungi which might be morphologically confused with them. The studies showed that the slant extracts were as useful as the culture filtrates and allowed more rapid identification of *C. immitis* and *H. capsulatum*. In every case, when an identification was made by conventional morphological methods, the immunological test yielded correlating results. The immunological tests were positive for two isolates that were converted to their yeast forms only after 4 months of study by conventional tests. The new procedure permits the identification of *C. immitis* and *H. capsulatum* 2 days after receipt of a pure mycelial-form culture. The test is recommended for the presumptive identification of *C. immitis* and *H. capsulatum* cultures.

Recently Standard and Kaufman (5, 6) reported using immunodiffusion and counterimmunoelectrophoresis procedures for the rapid detection of specific cell-free antigens produced by cultures of *Coccidioides immitis* and *Histoplasma capsulatum* grown in brain heart infusion broth. Recognition of these antigens permitted the identification of isolates of *C. immitis* and *H. capsulatum* within 5 days of receipt of pure cultures instead of the 3 weeks or longer frequently required for identification by conventional morphological procedures.

The present study was undertaken to determine whether the specific exoantigens of *C. immitis* and *H. capsulatum* could be extracted directly from agar slants of purified isolates. Such extraction would eliminate the need to use filtrates of brain heart infusion broth subcultures as a source of exoantigens. Development of such a technique might permit the accurate immunological identification of suspected isolates of these fungus pathogens within a shorter period than the 5 days required by the previous procedure.

MATERIALS AND METHODS

Unknown cultures. Duplicate purified cultures of a variety of fungi suspected of being *C. immitis* or *H. capsulatum* were received from the Fungus Reference Laboratory, Mycology Division, Center for Disease Control (CDC), Atlanta, Ga.

These specimens, in their mycelial form, were growing on Sabouraud dextrose agar (Difco) slants in tubes 18 by 150 mm.

Test procedure. Each culture was tested for specific exoantigens by the method of Standard and Kaufman (5, 6) and by a simpler modified procedure. The latter procedure was as follows: 7- to 10-day-old Sabouraud dextrose agar slant cultures with at least 15 by 30 mm of growth were covered with 8 to 10 ml of a 1:5,000 solution of Merthiolate and phosphate-buffered saline (pH 7.2). This solution was permitted to react with the cultures for 24 h at 25°C. The effectiveness of Merthiolate as a fungicide was verified in the preliminary studies. A 5-ml sample of each sterile cellular extract was then transferred to an Amicon Minicon macrosolute B-15 concentrator (Amicon Corp., Lexington, Mass.). Extracts derived from cultures suspected of being *C. immitis* were concentrated 25×. Those from cultures suspected of being *H. capsulatum* were concentrated 50×. The concentration process took approximately 2.5 h. The concentrated extracts were tested for exoantigens in the 24-h microimmunodiffusion test (5, 6). A fungus isolate was identified as *C. immitis* when its concentrated extract or culture filtrate was demonstrated to contain antigens identical to either the tube precipitinogen, the heat-labile precipitinogen, or the heat-labile F precipitinogen (2, 6), and was identified as *H. capsulatum* when H or H and M precipitinogens were produced (5).

Control reagents. Coccidioidin (CDC lot 1) and histoplasmin (CDC lot 9) were used as reference antigens. Sera from a patient with coccidioidomycosis (CDC lot 18311) and from a patient with histoplasmosis (CDC lot 10716) were used as test antisera.

RESULTS

Unknown coded cultures of hyphomycetes obtained from the Fungus Reference Branch of the Mycology Division, CDC, were tested for *C. immitis* or *H. capsulatum* exoantigens. The type of reagent applied was designated by the Fungus Reference Laboratory and depended upon a variety of factors such as whether the fungus bore macroconidia, tuberculate or smooth; the presence of arthroconidia; the clinical suspicions of the physician; and the geographic location of the patient from whom the culture was originally isolated. The results of microimmunodiffusion tests for *H. capsulatum* and *C. immitis* exoantigens in concentrated extracts and in culture filtrates are presented in Table 1, along with the identification of these isolates as determined by the conventional morphological procedures used in the Fungus Reference Branch.

It is apparent from the data in Table 1 that, regardless of whether the slant extract or the culture filtrate procedure was used, the *C. immitis* or *H. capsulatum* isolates were accurately identified. Of 32 isolates of *C. immitis* and *Histoplasma* spp. tested, all were found to be antigenic, and their identification was based upon production of their specific precipitinogens. The immunological results correlated fully with those obtained by the conventional morphological studies.

It should be noted that the slant extract procedure did not permit maximum formation of the diagnostic precipitinogens. Many isolates that produced two to three antigens by the culture filtrate procedure produced only one to two by the slant extract procedure.

From the data provided, it is apparent that many of the pathogenic isolates studied had been classified as atypical, i.e., nonsporulating or producing noncharacteristic smooth or echinulate macroconidia. The immunological procedures proved effective in correctly identifying these aberrant isolates.

Two isolates (no. 19 and 42) that produced H and M antigens converted to their yeast forms only after 4 months of serial subculture on a variety of media.

DISCUSSION

Our studies indicate that the slant extract procedure is as reliable and sensitive for identifying cultures of *C. immitis* and *H. capsulatum* as the culture filtrate procedure. Use of the slant extracts simplifies the identification procedure by eliminating the transferring of cultures and the use of additional media, a shake apparatus, and centrifugation required in the culture filtrate procedure. Most important of all, it eliminates

the need to work with the viable cultures of potentially dangerous isolates used in the culture filtrate method. The modified procedure shortens the time necessary to accomplish the immunological identification of *C. immitis* and *H. capsulatum* from 5 to 2 days after a pure mycelial-form culture is received. Our experiences also indicate that antigen extracted from the agar slants with water containing 1:5,000 Merthiolate is comparable to that obtained with a 1:5,000 solution of Merthiolate and phosphate-buffered saline (pH 7.2).

Conventional morphological identification of these fungi requires that their dimorphic nature be demonstrated. Our earlier studies (5, 6) revealed that this was not always easy to accomplish. In fact, in the present study, two *H. capsulatum* isolates (no. 19 and 42) were converted only after 4 months of growth at 37°C in vitro. Kwon-Chung and co-workers (3, 4) have shown that isolates of *Emmonsia capsulata* (*H. capsulatum*), particularly those of the + mating type, do not readily convert to the unicellular form in vitro. Such isolates, however, may be converted by the more time-consuming in vivo procedures. The exoantigen test provides an excellent means for identifying isolates of *H. capsulatum* that resist conversion. In addition to being rapid, the test provides presumptive results that so far have been found to be as reliable as the morphological conversion test results for identifying cultures of *C. immitis* and *H. capsulatum*. Furthermore, the exoantigen test makes possible the presumptive identification of *C. immitis* or *H. capsulatum* cultures whether they are in a sporulating or a nonsporulating state. Invariably, *H. capsulatum* formed H or M precipitinogens and *C. immitis* formed tube, heat-labile, or F precipitinogens. Detection of these antigens can be accepted as presumptive evidence of the identity of such isolates. Accordingly, we recommend the use of the exoantigen test for the rapid and accurate presumptive identification of the mycelial forms of cultures suspected of being *C. immitis* or *H. capsulatum*. Widespread use of these tests would be dependent upon the availability of specific antisera. This should pose no problem. The H and M precipitins may be produced in rabbits according to the method of Green et al. (1), whereas the tube, heat-labile, and heat-labile F precipitins have been successfully produced by us with the method described by Standard and Kaufman (5).

Since the submission of this paper for publication (February 1978), we have tested an additional 140 *H. capsulatum*, 120 *C. immitis* and 50 *B. dermatitidis* isolates and 181 morphologically similar hyphomycete cultures. The results

TABLE 1. Results obtained for 45 unknown hyphomycetes with the slant extraction procedure for identifying *C. immitis* and *Histoplasma* spp. and with the conventional immunological and morphological procedures

Culture no.	Conidia produced ^a	Precipitinogens in ^b :		Identification by conventional tests
		Extract	Culture filtrate	
1	Arthroconidia	TP, F	TP, HL, F	<i>C. immitis</i>
2	Atypical	H	H, M	<i>H. capsulatum</i>
3	Atypical	TP, HL	TP, HL, F	<i>C. immitis</i>
4	Arthroconidia	TP, HL	TP, HL, F	<i>C. immitis</i>
5	Tuberculate ma-croconidia	H, M	H, M	<i>H. capsulatum</i>
6	Arthroconidia	O	O	Saprophyte
7	Atypical	TP, HL	TP, HL, F	<i>C. immitis</i>
8	Atypical	TP	TP, HL	<i>C. immitis</i>
9	Atypical	H	H, M	<i>H. capsulatum</i>
10	Tuberculate ma-croconidia	H, M	H, M	<i>H. capsulatum</i>
11	Atypical	O	O	<i>Sepedonium</i> sp.
12	Atypical	TP	TP, HL	<i>C. immitis</i>
13	Atypical	O	O	<i>Chrysosporium</i> sp.
14	Arthroconidia	TP, HL	TP, HL	<i>C. immitis</i>
15	Atypical	TP	TP, HL	<i>C. immitis</i>
16	Atypical	TP	TP, HL	<i>C. immitis</i>
17	Atypical	TP	TP, HL, F	<i>C. immitis</i>
18	Atypical	TP, HL	TP, HL	<i>C. immitis</i>
19	Tuberculate ma-croconidia	H, M	H, M	<i>H. capsulatum</i>
20	Arthroconidia	O	O	<i>Malbranchea</i> sp.
21	Arthroconidia	TP	TP, HL, F	<i>C. immitis</i>
22	Arthroconidia	TP, HL, F	TP, HL, F	<i>C. immitis</i>
23	Atypical	TP	TP, HL	<i>C. immitis</i>
24	Arthroconidia	O	O	<i>Auxarthron</i> sp.
25	Arthroconidia	TP	TP	<i>C. immitis</i>
26	Arthroconidia, chlamydospores	TP	TP, HL	<i>C. immitis</i>
27	Atypical	TP	TP, HL	<i>C. immitis</i>
28	Arthroconidia	TP, HL, F	TP, HL, F	<i>C. immitis</i>
29	Arthroconidia	TP, F	TP, HL, F	<i>C. immitis</i>
30	Arthroconidia	TP, F	TP, HL, F	<i>C. immitis</i>
31	Atypical	TP	TP, HL, F	<i>C. immitis</i>
32	Arthroconidia	O	O	<i>Malbranchea</i> sp.
33	Tuberculate ma-croconidia	H, M	H, M	<i>H. capsulatum</i>
34	Atypical	O	O	<i>B. dermatitidis</i>
35	Arthroconidia	TP, HL, F	TP, HL, F	<i>C. immitis</i>
36	Arthroconidia	O	O	<i>Auxarthron</i> sp.
37	Arthroconidia	O	O	<i>Arachniotus</i> sp.
38	Atypical	H, M	H, M	<i>H. capsulatum</i> subsp. <i>du-boisii</i>
39	Atypical	O	O	<i>Chrysosporium</i> sp.
40	Tuberculate ma-croconidia	H, M	H, M	<i>H. capsulatum</i>
41	Atypical	O	O	<i>C. keratinophilum</i>
42	Tuberculate ma-croconidia	H, M	H, M	<i>H. capsulatum</i>
43	Atypical	O	O	<i>Sepedonium xylogenum</i>
44	Atypical	H, M	H, M	<i>H. capsulatum</i> subsp. <i>du-boisii</i>
45	Atypical	O	O	<i>Arthroderma tuberculatum</i>

^a Atypical sporulation, culture demonstrates either no macroconidia or smooth or echinulate macroconidia.^b *C. immitis* F antigen; H, *H. capsulatum* H antigen; HL, *C. immitis* heat-labile antigen; M, *H. capsulatum* M antigen; O, negative; TP, *C. immitis* tube precipitinogen.

obtained were in complete agreement with those obtained previously and described in this report.

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