APPLIED MICROBIOLOGY, Mar. 1972, p. 490-499
Vol. 23, No. 3

Comparison of a Newly Developed Latex Agglutination Test and an Immunodiffusion Test in the Diagnosis of Systemic Candidiasis[1]

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Received for publication 13 October 1971

A newly developed latex agglutination (LA) test and a modified immunodiffusion (ID) test were evaluated. The antigen used was a homogenate of *Candida albicans*. A total of 167 antisera were employed in the evaluation. They included 36 sera from clinically well persons; 78 from patients with various clinical forms of candidiasis; 52 from patients with proven cases of aspergillosis, blastomycosis, coccidioidomycosis, cryptococcosis, histoplasmosis, nocardiosis, paracoccidioidomycosis, sporotrichosis, and tuberculosis; and one serum from a patient with torulopsosis. Use of the LA test in conjunction with the ID test permitted the detection of more than 90% of 43 proven candidiasis cases. Of all the heterologous cases and normal human sera tested, LA reactions were noted with 3 of 10 cryptococcosis case specimens, 1 of 9 tuberculosis case specimens, and with the torulopsemia case serum. In contrast, the only heterologous serum reactive in the ID test was that from the patient with torulopsemia. *Torulopsis glabrata* and *C. albicans* antisera gave identical reactions in LA and ID tests with *T. glabrata* or *C. albicans* antigens. ID tests with selected antigens, however, permitted differentiation of rabbit and human *T. glabrata* antibody from that of *C. albicans* antibody. Six different precipitins were recognized with the *C. albicans* antigens. The occurrence of multiple precipitin lines and high LA titers was suggestive of severe candidiasis. The LA test, in contrast to the ID test, appeared to have prognostic value. Together, the LA and ID tests provided a simple, rapid, and accurate means of detecting and monitoring infections by species of *Candida*.

During recent years, the prevalence of systemic candidiasis has increased in patients who experienced either abdominal surgery, immunosuppressive, extensive antibiotic, or adrenal cortical steroid therapy. Control and treatment of this opportunistic disease are dependent upon its early and accurate diagnosis. Unfortunately, visceral candidiasis lacks unique clinical features, and the diagnosis is usually not made until autopsy (9).

Candidiasis may be diagnosed by the repeated isolation of a *Candida* species in what are normally sterile clinical specimens, such as blood and spinal fluid. Because species of *Candida* normally occur throughout the gastrointestinal tract as commensals, the significance of their isolation from the mouth, sputum, urine, or stool is often difficult to evaluate. However, repeated isolation of a *Candida* species in the absence of other pathogens may implicate the isolate as the etiologic agent of the disease. Although histological evidence is definitive, it is often difficult to obtain. Diagnosis is also frequently delayed by the fact that the isolation and identification of *Candida* species from clinical materials may require 2 or more weeks to accomplish.

Hypersensitivity to *Candida* skin test antigens is not of diagnostic value, since 83% of apparently healthy individuals give positive reactions (9). Positive reactions with serological tests other than the immunodiffusion test have also been inconclusive. Up to 89% of apparently healthy people have antibodies in their sera that can agglutinate *C. albicans* (3, 6, 17). Similar difficulties have been experienced with sera from apparently healthy humans with complement fixation (7), hemagglu-

[1] A portion of a dissertation submitted by the senior author to the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Public Health in the School of Public Health.

tination (M. Goldin, Bacteriol. Proc., p. 106, 1957), and the indirect fluorescent-antibody tests (4).

Stallybrass (11) and Taschdjian et al. (14), using the immunodiffusion (ID) technique, demonstrated precipitating antibodies against *C. albicans* in sera from patients with systemic or visceral *Candida* infections. In contrast, sera from healthy persons or patients with mucocutaneous candidiasis did not contain such antibodies. Furthermore, contrary to results with other serological reactions, the precipitin test appeared to be free from cross-reactions, except among species of the genus *Candida* (15).

Although the ID test appears to be the most useful method for the serological detection of systemic candidiasis, it is essentially qualitative and takes days to perform. Changes in antibody titers which range from 1:1 to 1:4, moreover, do not reflect the effect of therapy (16).

Amphotericin B is the drug of choice in the treatment of systemic candidiasis. Its use entails the risk of dangerous side effects, most notably nephrotoxicity. Consequently, the use of amphotericin B should be based upon reliable diagnostic and prognostic tests.

The purpose of this study was to develop a quantitative latex agglutination (LA) test or a modified ID test that would detect cases of systemic candidiasis and also permit accurate monitoring of the course of the disease. In addition, we wished to characterize the precipitin response and to relate it to the clinical state of the patient.

MATERIALS AND METHODS

Cultures. The cultures consisted of *C. albicans*, type A, B385; *C. albicans*, type B, B1074; *C. guilliermondii* B1075; *C. krusei* B1076; *C. parapsilosis* 172; *C. pseudotropicalis* B1080; *C. stellatoidea* B1017; *C. tropicalis* B1081; and *Torulopsis glabrata* B989. Appropriate physiological and morphological tests were performed to verify the identity of all of the fungi used in this study (1).

Antigen preparation. Whole cell antigens were prepared from 48-hr dextrose-peptone-yeast extract broth cultures grown at 37 C by the method of Sweet and Kaufman (12). Working suspensions were prepared by diluting one part of packed cells in three parts of 0.5% formalinized saline and stored at 4 C. These antigens were used for (i) production of antisera, (ii) preparation of homogenates, and (iii) agglutination tests.

Antisera production. Rabbits receiving a total of 8.5×10^7 cells were immunized with *Candida* species and *T. glabrata* by the procedure of Sweet and Kaufman (12). Except for the *C. pseudotropicalis* antisera, which showed tube agglutinin titers of 1:64, all rabbit antisera showed agglutinin titers ranging from 1:512 to 1:2,048.

Homogenate antigens. Fifty grams of glass beads (0.5 mm) was placed in 60-ml, screw-capped serum bottles. The bottles and beads were washed in 1% HCl, rinsed in distilled water, and chilled in the freezer before being used. A 20-ml amount of the 1:4 cell suspensions was then added to the beads and disrupted with a Braun "MSK" mechanical cell homogenizer. The homogenizer was run for 2-min intervals after which the extent of cell disruption was determined by microscopic examination. Over 90% of the blastospores were disrupted in 10 min. The homogenates were centrifuged for 30 min at 3,000 $\times$ *g*. Supernatant fluids were pooled, refrigerated overnight at 4 C, and centrifuged again. The final supernatant fluids were collected and concentrated by pervaporation at 4 C or with Amicon PM 10 membranes to a total biuret protein concentration of 1.0 g per 100 ml. Merthiolate was added to a concentration of 1:10,000, and the homogenate (H) antigen was stored at 4 C.

Commercial antigens. Lots of Hollister-Stier (HS) 1:10 extracts of *C. albicans* in 50% glycerine-saline solution were used (Hollister-Stier Laboratories, Spokane, Wash.). Various lots demonstrated a total biuret protein concentration of between 0.8 and 1.0 g per 100 ml.

ID tests. ID tests were performed to detect antibodies to *Candida* species and *T. glabrata*. The test medium consisted of 0.9% agarose (Colab Laboratories, Inc.) in 100 ml of phosphate-buffered saline (*p*H 7.2; PSB) containing 1.0 ml of 1.0% Merthiolate. A 7-ml amount of the above agar was pipetted onto 2- by 3-inch slides (5.08 by 7.62 cm). Using the method of Piazzi (8), we developed a template that incorporated antigen-antibody wells of such diameter that they permitted detection of optimal combining proportions of reactants. This template indicated optimal sensitivity for reactions of rabbit and human serum with a serum to antigen ratio of 4 (8-mm well) to 3 (6-mm well). The standard pattern consisted of three serum wells and four antigen wells; the reactant reservoirs were placed at a distance of 10 mm. Box titration with rabbit antisera produced from the seven medically important *Candida* species and proven human candidiasis case sera indicated the optimal dilution of the H and HS antigens to be 1:5. The optimal antigen dilution was the highest dilution giving the most precipitin lines with each of the *Candida* species antisera. The slides with charged wells were incubated at 25 C in a moist chamber and examined daily for 3 days. All precipitin bands were characterized by relating them to bands produced by rabbit anti-*C. albicans* sera or selected human candidiasis case sera.

LA test. LA tests were performed by a modification of the method of Bloomfield et al. (2). Specimens of serum were inactivated at 56 C for 30 min and then serially diluted in *p*H 9.0 glycine-buffered saline (GBS) containing 0.1% bovine serum albumin. Equal volumes of a standardized suspension of latex particles were sensitized with a concentration of H (5$\times$) or HS (20$\times$) antigen which gave a maximum

agglutination reaction in box titrations against selected rabbit *C. albicans* antiserum. Latex and antigen were allowed to interact for 30 min at 25 C. Fifteen volumes of GBS were added, and the washed suspensions were sedimented by centrifugation for 30 min at 3,000 × *g*. The sensitized latex particles were then restored to the original volume with GBS.

Test serum (0.02 ml) was mixed with 0.02 ml of the latex-antigen suspension on a glass slide and agitated for 5 min on a rotary shaker at 160 rev/min. Agglutinations of 1+ (fine granular agglutination against a milky background) or greater were recorded as positive. The antibody titer was recorded as the highest dilution of serum which gave an agglutination equivalent to the 1+ positive control. Two control sera were an essential part of each test: (i) a positive serum with a 1+ reaction, and (ii) a nonreactive serum.

Sera. Specimens received in the Fungus Immunology Unit, Center for Disease Control, were from patients with culturally proven cases of systemic mycotic and bacterial infections and from apparently healthy humans. The clinical diagnosis in each case was obtained from the attending physician. All sera were inactivated by heating at 56 C for 30 min.

RESULTS

Antigen evaluation. The quality of the H and HS antigens was first evaluated in ID tests. Table 1 shows the results of titrations of these two *C. albicans* antigens with rabbit antisera produced from the seven medically important *Candida* species. The optimal dilution was found to be 1:5 for both the H and HS antigens. However, as shown, the H antigen gave more bands with *C. albicans* (A) and *C. tropicalis* antisera than the HS antigen.

Since the H antigen gave the greater number of bands, its spectrum of reactivity with the *Candida* species antisera was examined closely; *C. albicans* and *C. tropicalis* antisera gave three bands with this antigen, and antisera to the remaining *Candida* species, except *C. krusei*, gave one band of identity. *C. krusei* antiserum was unique in that it gave no precipitin bands with the *C. albicans* H antigen but did react with its homologous antigen. *C. parapsilosis* was also unique, because its precipitin was not identical with the precipitins produced by other *Candida* species.

A striking serological similarity between *C. albicans* (A) and *T. glabrata* antisera was noted (Fig. 1). Reactions with homologous and heterologous antigens produced three lines of identity. All three *C. albicans* precipitin bands formed with homologous *C. albicans* antisera were exhibited by *T. glabrata* antisera. Likewise, *T. glabrata* antigen elicited identical bands with *C. albicans* antisera.

Nevertheless, adsorption studies revealed subtle differences between these two antigens. *C. albicans* antisera, adsorbed with *T. glabrata* antigen, reacted with *C. albicans* antigen but not with *T. glabrata* antigen. *T. glabrata* antisera, adsorbed with *C. albicans* antigen, reacted with *T. glabrata* antigen and not with *C. albicans* antigen.

Since adsorption procedures are not amenable to routine serological testing a simplified

TABLE 1. *Titration of the homogenate (H) and Hollister-Stier (HS) antigens of Candida albicans with rabbit antisera produced from whole cell antigens of eight Candida species*

Antisera	No. of precipitin lines with antigen dilution							
	H antigen				HS antigen			
	1:1	1:2	1:5	1:10	1:1	1:2	1:5	1:10
C. albicans (A)	3	3	3	2	2	2	2	1
C. albicans (B)	1	1	1	0	1	1	1	0
C. guilliermondii	1	1	1	1	1	1	1	1
C. krusei	0	0	0	0	0	0	0	0
C. parapsilosis	1	1	1	0	1	1	1	0
C. pseudotropicalis	1	1	1	0	1	1	1	0
C. stellatoidea	1	1	1	0	1	1	1	0
C. tropicalis	3	3	3	2	2	2	2	1

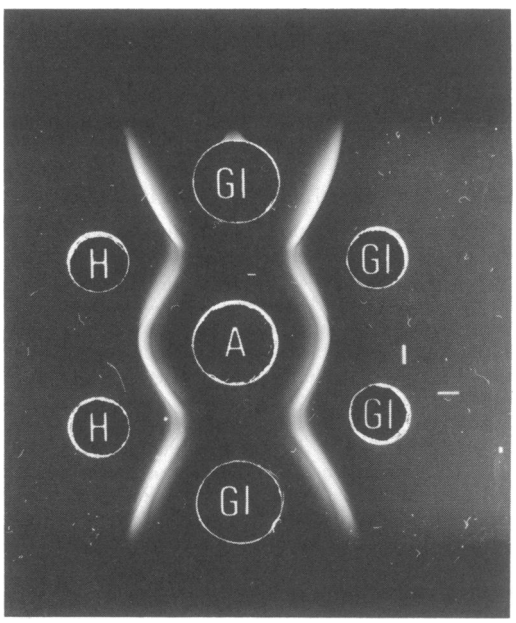

FIG. 1. *Precipitin lines produced by Torulopsis glabrata (GI) and Candida albicans (A) rabbit antisera and their homologous and heterologous homogenate antigens (GI and H); antigens are in small wells.*

method of delineating *C. albicans* antibody from *T. glabrata* antibody was sought. Figures 2 and 3 show the differences between unadsorbed *C. albicans* and *T. glabrata* antisera interacting with *C. tropicalis* and *T. glabrata* antigens. *C. albicans* antisera gave a line of nonidentity with *C. tropicalis* and *T. glabrata* antigens. The smooth line of identity between *C tropicalis* and *T. glabrata* antigens when reacted with *T. glabrata* antisera demonstrates the difference between *C. albicans* and *T. glabrata* antisera. In addition, *T. glabrata* antisera reacted with *C. krusei* antigen, whereas *C. albicans* antisera invariably did not. Comparative reactions of *C. albicans* and *T. glabrata* antisera with *C. krusei* antigen are shown in Fig. 4.

Latex agglutination tests. The effectiveness of the *C. albicans* antigens in LA tests was ascertained by box titrations with rabbit *C. albicans* antisera, demonstrating an agglutinin titer of 1:1,024. The 10× concentrated H antigen and the 40× concentrated HS antigen both demonstrated optimal antigen activity at the 1:2 dilution. Rabbit antisera prepared against the medically important *Candida* spe-

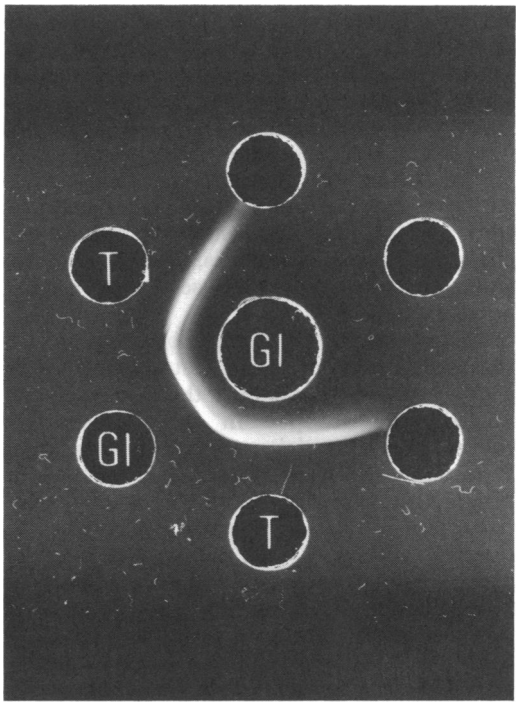

FIG. 3. *Precipitin lines produced by Torulopsis glabrata rabbit antiserum (G1) and homogenate antigens of Candida tropicalis (T) and T. glabrata (G1); antigens are in small wells.*

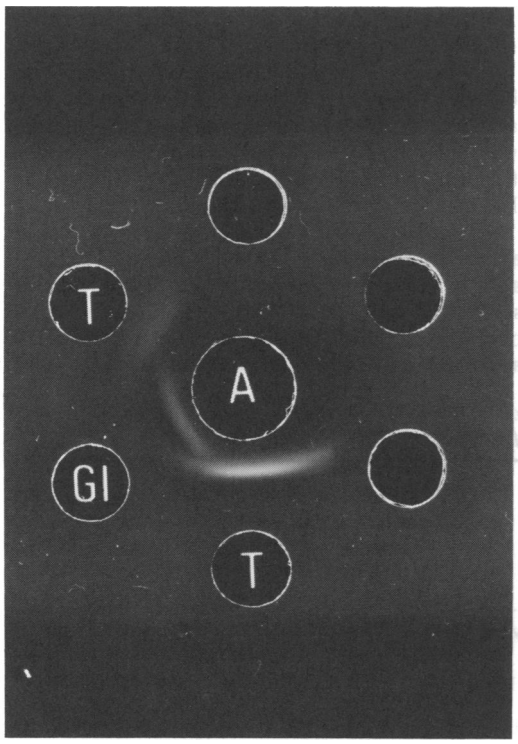

FIG. 2. *Precipitin lines produced by Candida albicans rabbit antiserum (A) and homogenate antigens of C. tropicalis (T) and T. glabrata (G1).*

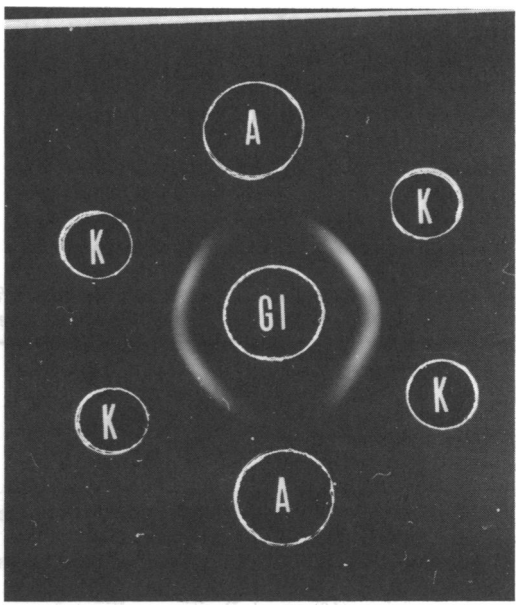

FIG. 4. *Comparison of precipitin reactions with Candida albicans (A) and Torulopsis glabrata (G1) rabbit antisera against C. krusei homogenate antigen (K).*

cies were titrated with the optimally diluted H and HS antigens in LA tests. Latex particles sensitized with both antigens reacted with rabbit antisera to all of the *Candida* isolates studied. Low but positive titers were obtained with the *C. krusei* antisera.

Evaluation with human sera. Proper evaluation of the LA and ID procedures required tests with sera from patients with the various clinical forms of candidiasis, related mycoses, and tuberculosis and with sera from normal humans.

Table 2 shows the results obtained with 78 candidiasis sera in LA and ID tests with the H antigen. Cases from which the sera were obtained were grouped in three categories: proven, highly suspicious, and least suspicious. Proven case sera were those from patients with histological evidence of candidiasis or with deep tissue biopsies positive by culture for *Candida* species. All of the isolates were *C. albicans* except for one which was *C. parapsilosis*. Highly suspicious sera were those from patients with repeatedly positive blood cultures, whereas sera in the least suspicious category were from patients with positive cultures from various body fluids, which normally may contain *Candida* species.

The LA test detected 95% (41/43) of the proven cases. In contrast, the ID test detected 88% (38/43). In the highly suspicious category, the diagnosis was aided in 79% (15/19) of the cases by the LA test and in 63% (12/19) by the ID test. Sixty-three and 31% of the cases in the least suspicious category were serologically positive by the LA and ID tests, respectively.

Table 3 shows LA and ID results for 67 sera from 63 proven or highly suspected candidiasis cases. A few sera represented serial specimens taken at various intervals during the patients' illness. The sera were categorized according to the patient's clinical manifestations as noted by the attending physician. They were then related to the number of ID bands produced and to the LA titer. The data show that multiple bands (three or more) were found most often in disseminated candidiasis and in *Candida* endocarditis. These two clinical states were also associated with the greatest proportion of sera demonstrating LA titers of 1:8 or greater. Of the false-negative reactions obtained with both tests, nearly half were with sera from cases of pulmonary and superficial candidiasis.

Since we noted that ID tests with various human candidiasis case sera revealed either single or multiple bands, we characterized the types of precipitin reactions. Figure 5 shows a typical negative ID test. A positive control reference rabbit *C. albicans* antiserum is in the large middle well. The top and bottom center wells contain negative human serum. The small wells contain the H antigen. Three precipitin lines designated a, b, and c are typically obtained with the rabbit control serum. (The line designated "a" is the one closest to the serum well.)

Figure 6 shows a strongly positive reaction of a human serum (Nc); the precipitin line corresponds to the b band of the rabbit control (A). This human serum was especially interesting because there were two bands between its well and the rabbit antiserum well. This reaction was thought to be due to rheumatoid factor; this assumption was confirmed by a positive LA test for rheumatoid factor.

As shown in Table 3, most of the sera tested exhibited from one to three bands identical to a, b, or c bands produced by the rabbit control sera. Five candidiasis case sera, however, gave one or three additional bands not identical to a, b, or c that were arbitrarily labeled d, e, and

TABLE 2. *Latex agglutination (LA) and immunodiffusion (ID) results obtained with sera from 78 culturally positive cases of candidiasis*

Case category (no. of specimens)	Criteria	No. of Specimens				Per cent sensitivity	
		Positive		Negative		ID	LA
		ID	LA	ID	LA		
Proven (43)	Histological evidence, biopsy culture positive	38	41	5	2	88	95
Highly suspicious (19)	Blood cultures consistently positive	12	15	7	4	63[a]	79[a]
Least suspicious (16)	Positive culture from multiple body fluids: sputum, urine, wound drainings	5	10	11	6	31[a]	63[a]

[a] Sensitivity here is based on the assumption that these are all cases of systemic candidiasis.

TABLE 3. *Latex agglutination (LA) and immunodiffusion (ID) reactions with 67 sera from 63 candidiasis cases categorized according to the patients clinical manifestations*

Clinical manifestation (no. of specimens)	Serological reactions by no. of specimens													
	ID test positive (no. of bands)						ID test negative	Reciprocal of LA titer						LA test negative
	1	2	3	4	5	6		1	2	4	8	16	32	64	
Systemic candidiasis															
Disseminated (9)	1	1	1			3	3		1	2	3	1			2
Septicemia (11)	6	2					3	6	1	2					2
Pulmonary (11)	5						6		1	4		1	1		4
Kidney (6)	3	3						2		2	1	1			
Gastrointestinal tract (5)	2	1	1				1		2	2			1		
Endocarditis (16)		8	5	2			1	6		1	4	2	2	1	
Superficial candidiasis															
Mucous membranes (9) (Skin, thrush)	1	2					6	1		1	3				4

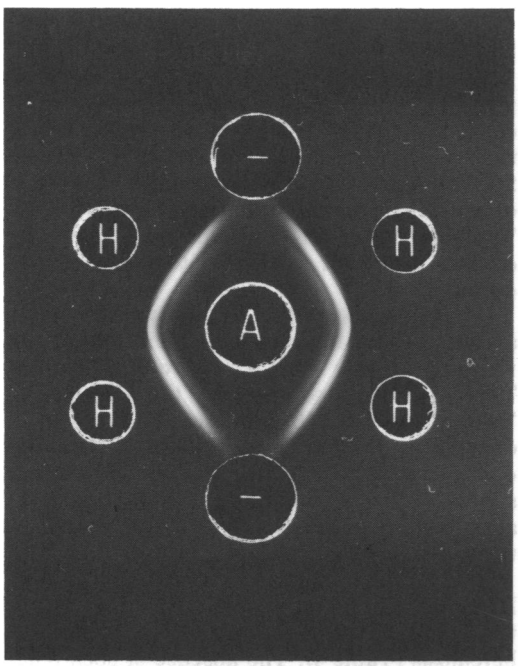

FIG. 5. *Three precipitin lines produced by rabbit Candida albicans control serum (A) against the homogenate (H) antigen of C. albicans. The a band is closest to the serum well and the c band is closest to the antigen well.*

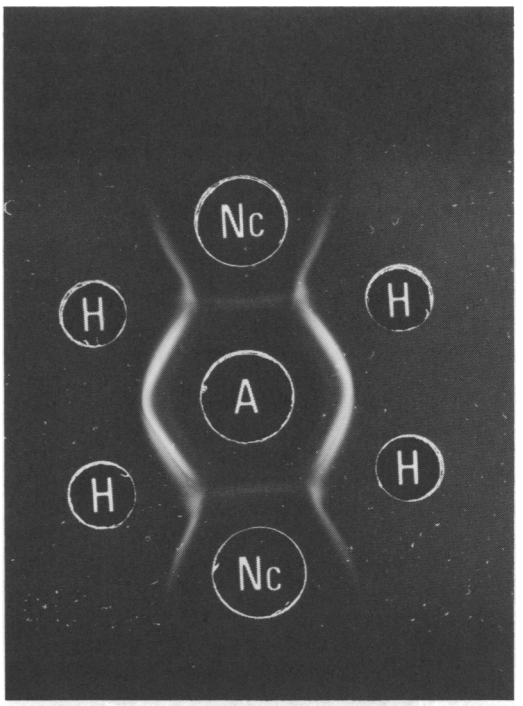

FIG. 6. *Comparison of the precipitin line produced by a positive human candidiasis case serum (Nc) with the lines produced by the control rabbit Candida albicans serum (A) reacting with the homogenate (H) antigen of C. albicans.*

f. These precipitins were not in the rabbit serum. Various combinations of these bands occur. Figure 7 shows two human sera that revealed only two of these extra bands. Serum E exhibits only d and e bands, and serum G exhibits the b and e bands.

The LA test may be of prognostic value. Studies of serial serum specimens from two candidiasis patients showed that latex agglutination titers decreased in one case from 1:16 to 0 and in a second case from 1:32 to 1:1 after amphotericin B treatment. Two precipitin bands in case one remained after treatment, whereas two of three precipitins were retained in the serum of case 2. The decreases in LA titer correlated with the cure of both

patients as revealed by clinical improvement and by subsequent failure to isolate candidas.

Table 4 shows the LA and ID test results obtained with sera from 66 proven cases of related mycoses, tuberculosis, and unrelated diseases and from 36 normal humans. Three of 10 cryptococcosis case sera and 1 of 9 tuberculosis case sera gave positive results with the LA test, but these sera were not positive with the ID test. However, one serum from a proven case of torulopsemia was positive with both the LA and ID tests. The ID test demonstrated a specificity of 99%, and the LA test demonstrated a specificity of 95% with the 102 heterologous case and normal sera used in this study.

Although *T. glabrata* infections are rare and are treated the same as candidiasis, the need could arise for differentiating the two diseases serologically. Figures 8 and 9 demonstrate the serological differentiation of human *C. albicans* case serum from a *T. glabrata* human case serum. When the *C. albicans* case serum reacted with *C. tropicalis* and *T. glabrata* antigens placed side by side, lines of nonidentity were produced. These results are similar to those obtained with rabbit *C. albicans* antisera (Fig. 2 and 3). When tested similarly, however, human serum from a case of torulopsemia produced a line of identity after reaction with *C. tropicalis* and *T. glabrata* antigens. Similar results had been obtained with rabbit *T. glabrata* antisera. The human *T. glabrata* serum also reacted with *C. krusei* antigen, whereas the human *C. albicans* serum did not.

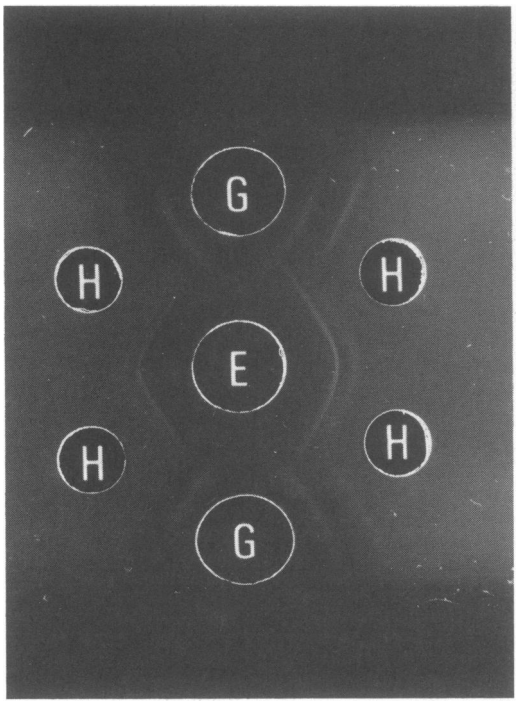

FIG. 7. *Demonstration of the additional precipitin lines produced by two human candidiasis case sera (G and E) and the homogenate (H) antigen of Candida albicans.*

DISCUSSION

Our studies indicate that LA and ID tests are valuable in the diagnosis of systemic candidiasis. Studies with rabbit antisera to the seven medically important *Candida* species indicate that antibodies to all of these pathogens react with latex particles sensitized with H or HS *C. albicans* antigens. Similar studies with ID tests revealed that H and HS precipitinogens react with antibodies to only six of the *Candida* species, the exception being antibodies of *C. krusei*.

Parallel studies with 43 sera from patients with proven human systemic candidiasis indicated that the LA test has a greater sensitivity than the ID test, 95% in contrast to 88% (Table 2). The sensitivity of our modified ID test is essentially similar to that obtained by Taschdjian et al. (15), who demonstrated precipitins in 85% of patients with systemic candidiasis.

Of the two tests the ID test was the most specific (Table 4). Extrageneric cross-reactions were noted only with *T. glabrata* antiserum. This finding is contrary to the findings of Taschdjian et al. (16), who noted no cross-precipitins in *T. glabrata* antiserum. Otherwise, our studies with heterologous case and normal sera support the contention of Stallybrass (11) and Taschdjian et al. (15) that the precipitin test gives no false positives.

The LA test exhibited a specificity of 95%. Reactions were demonstrated in 3 of 10 patients with cryptococcosis and 1 of 9 with tuberculosis (Table 4). The absence of such reactions with rabbit *C. neoformans* antisera suggests that these reactions rather than being false positives might be specific and might reflect dual infections.

In our study, the homogenate or H antigen prepared from *C. albicans* cells gave the most satisfactory LA and ID results (Table 1). Nevertheless, the commercially available HS antigen was found to be a suitable substitute for use in both test procedures. Taschdjian et al. (13) reported similar results with their S antigen and with the HS antigen and recommended the latter as an acceptable substitute for the S antigen. Undoubtedly, the commer-

TABLE 4. *Results obtained with latex agglutination (LA) and immunodiffusion (ID) tests against 102 sera from normal humans and proven cases of mycotic and nonmycotic diseases*

Category	No. of sera	No. of specimens				Per cent specificity	
		Positive		Negative			
		ID	LA	ID	LA	ID	LA
Aspergillosis	7	0	0	7	7	100	100
Blastomycosis	5	0	0	5	5	100	100
Coccidioidomycosis	5	0	0	5	5	100	100
Cryptococcosis	10	0	3	10	7	100	70
Histoplasmosis	5	0	0	5	5	100	100
Nocardiosis	1	0	0	1	1	100	100
Paracoccidioidomycosis	5	0	0	5	5	100	100
Sporotrichosis	5	0	0	5	5	100	100
Torulopsemia	1	1	1	0	0	0	0
Tuberculosis	9	0	1	9	8	100	89
Unrelated diseases	13	0	0	13	13	100	100
Normal human sera	36	0	0	36	36	100	100
Totals	102	1	5	101	97	99	95

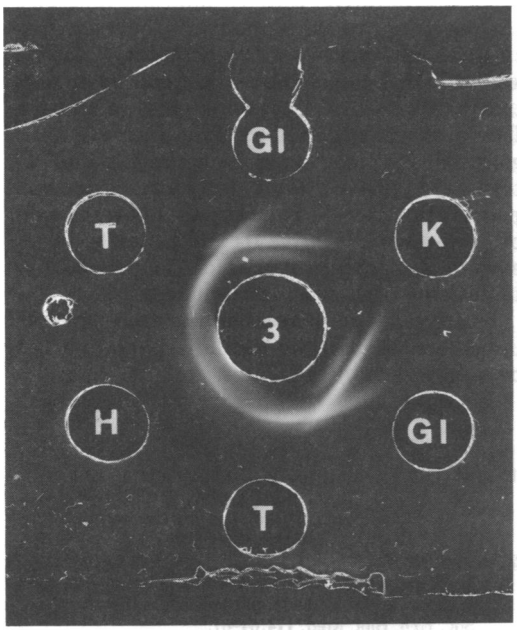

FIG. 8. *Precipitin lines produced by a human candidiasis case serum (3) against homogenate antigens of Candida albicans (H), C. tropicalis (T), C. krusei (K), and T. glabrata (G1).*

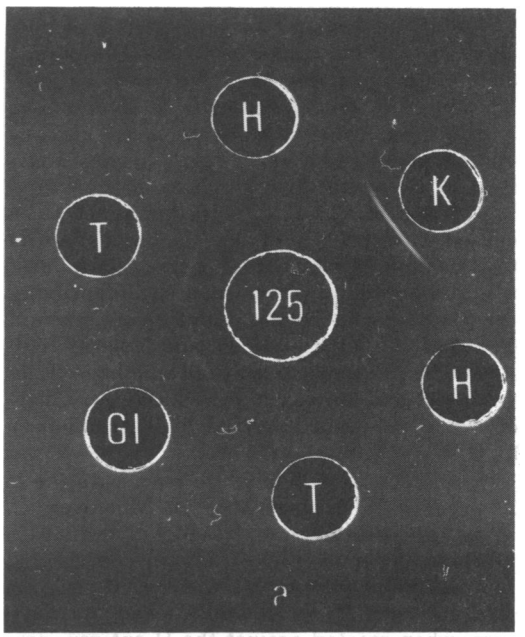

FIG. 9. *Precipitin lines produced by a human torulopsemia case serum (125) and homogenate antigens of Candida albicans (H), C. tropicalis (T), C. krusei (K), and T. glabrata (G1).*

cial availability of the HS antigen should lead to the widespread use of both the LA and ID tests.

In addition to being diagnostically applicable, the LA and ID tests show promise of having prognostic value. Serial specimens from three cases with progressive candidiasis exhibited higher LA titers as the disease process became progressively more severe. Titers increased from 1:1 in two cases and 1:8 in the third to 1:4, 1:16, and as high as 1:64 respectively, as the disease advanced. The LA test titers decreased after successful therapy with amphotericin B. Higher LA titers (1:16 or greater) were also found more often in the severe clinical manifestations of candidiasis (Table 3).

We have attempted to ascertain the signifi-

cance of the number and type of precipitins present in the sera of candidiasis patients. Enumeration of ID bands did not always permit a prediction of disease regression after successful treatment. This finding is contrary to the observations of Taschdjian et al. (16), who noted that precipitins disappear during the course of recovery from candidiasis. Knowledge of the identity of the precipitin bands produced in a patient's serum could be of value in recognizing cases of severe candidiasis. Our data indicate that the presence of more than three bands, or precipitins d, e, or f, is associated with severe disseminated candidiasis, endocarditis, or near terminal candidiasis (Table 3). This observation should be further investigated, since autopsy studies indicate that the detection of precipitins and isolation of *Candida* sp. from a variety of clinical materials fail to adequately indicate the extent or severity of the infection (10).

The ID test offers the opportunity of speciating one of the etiological agents of systemic candidiasis. Antisera to *C. albicans*, *C. guilliermondii*, *C. pseudotropicalis*, *C. stellatoidea*, and *C. tropicalis* produce lines of identity upon reaction with H antigen, whereas antisera to *C. parapsilosis* produce an unrelated line. A case of endocarditis was predicted to be caused by *C. parapsilosis* on the basis of an ID test on the patient's serum. Some 3 days later this prediction was confirmed by identifying the yeast species by the agglutination method of Sweet and Kaufman (12), and 3 weeks later it was confirmed by cultural methods. The unique precipitation band produced by *C. parapsilosis* has been described by Taschdjian et al. (15).

C. krusei antibody failed to react with the H antigen in ID tests (Table 1). Moreover, *C. krusei* antigen failed to react with rabbit *C. albicans* antibody (Fig. 4). *C. krusei* antibody, however, does react with *C. krusei* antigen.

C. albicans (type A) and *T. glabrata* antisera, when reacted against the H antigen, produced only lines of apparent identity (Fig. 1). Cross-adsorption of these two antisera, however, resulted in antibodies which reacted only with their homologous antigens. These findings are similar to those of Kemp and Solotorovsky (5), who performed similar adsorptions for studying *Candida* sp. and *T. glabrata* agglutinins. Because adsorption procedures are not amenable to routine serological testing, the simplified and effective method of differentiating candidiasis case sera from torulopsosis case sera with *C. tropicalis*, *T. glabrata*, and *C. krusei* antigens (Fig. 2 to 4) is recommended when torulopsosis is suspected.

With the H or commercially available HS antigen, the ID and LA tests may be used for the rapid and accurate screening of patients for evidence of systemic candidiasis. Antigens are stable for at least 6 months, a factor which enhances the reliability of day to day testing.

Both the LA and ID tests should be performed on suspected candidiasis case sera. A quantitative LA test result can easily be obtained in 15 min, and a positive LA test result can be confirmed by the ID test within 72 hr. A positive LA test result with a negative ID test result does not necessarily rule out a *Candida* sp. infection. Quantitative LA tests on sera taken at biweekly intervals may be of value in monitoring the progress of the infection before and after successful therapy. Increases in titer may indicate active disease.

Where candidiasis is suspected and ID reactions are negative with *C. albicans* antigens, the test could be performed with *C. krusei* antigen to rule out infection with this *Candida* species. This procedure is particularly important when the LA reaction is positive and the ID reaction is negative with the H or HS antigens. In addition, when both the LA and ID tests are positive and torulopsosis is suspected, tests for *T. glabrata* antibodies should be performed. Negative ID and LA tests do not exclude a diagnosis of candidiasis, particularly in patients with pulmonary disease.

Positive LA or ID tests appear to be specific indicators of systemic candidiasis and should be used widely by clinicians.

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