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Laboratory Evaluation of Serological Tests for Systemic Candidiasis: a Cooperative Study

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Three serological tests for candidiasis, agar gel diffusion (AGD-1), whole cell agglutination (AGGL-1), and latex agglutination (LAT), were evaluated by six laboratories with 100 coded sera. In addition, each of six laboratories performed a test of its choice, either the AGD-2, the AGD-3, the AGGL-2 or one of three counterimmunoelectrophoresis (CEP) methods (CEP-1, CEP-2, and CEP-3). Results are presented by laboratory for a group of 53 "candida-involved" cases (33 proven, 14 presumptive, and 6 probable) and 47 negative controls (41 normal and 6 other disease states). The AGD-1 test produced an overall average of 85.1% positive results in the candida-involved group and 5.0% positives in the control group. The LAT produced an overall average of 89.0% positives in the candida-involved group and 17.4% positives in the controls. The AGGL-1 test produced an overall average of 63.8% positives in the candida-involved group, with 12.3% positives in the controls. In the individual tests, the best performance was shown by the CEP-3 test (92.5% positives in the candida-involved group and 2.1% positives in controls) and the CEP-1 test (88.7% positive in the candida-involved group and no positives in the controls). The tests with the highest sensitivity were the AGGL-2 and CEP-2 (94.3 and 96.2%, respectively). These tests were also the least specific (80.9 and 76.6%, respectively). In the three common tests, the AGD-1 was the most reproducible, whereas the AGGL-1 produced considerable laboratory-to-laboratory variation. Since cell-free extracts of mechanically disrupted *C. albicans* were used for the LAT and all the AGD and CEP tests, the difference in performance was considered to be mainly due to antigenic composition and the conditions of the test. The results of this study confirm the value of serological tests for the diagnosis of systemic candidiasis, but point out the need for standardized reagents.

Despite the rising prevalence of systemic candida infection, antemortem diagnosis has been difficult to establish (24, 26, 30, 31). The potential threat of systemic candidiasis exists in patients who are chronically ill, who have undergone cardiac surgery, gastrointestinal surgery, or renal transplantation, or who have received intravenous catheters, hyperalimentation, or prolonged antibiotic or immunosuppressive therapy (3, 11, 21, 32, 37). Heroin addicts have been reported to have a high prevalence of systemic candidiasis diagnosed on postmortem examination (11, 35). Unfortunately, the criteria used to establish the diagnosis clinically have not been reliable. Demonstration of candida cells in tissues is pathognomonic, but tissue for antemortem histology can be difficult to obtain (11, 35, 18). The treatment of choice is amphotericin B or 5-fluorocytosine. The toxicity of amphotericin B and the discomfort it gives to patients preclude its use without a very high suspicion or established diagnosis of invasive candidiasis. For these reasons, a reliable serological method for early diagnosis is needed.

Development of a reliable serological method for the diagnosis of systemic candidiasis must take into account the antigenic complexity of *Candida albicans* (2). In most methods, serotype A isolates are used since they are more common than type B (13, 14). Other *Candida*

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species may be pathogenic, but *C. albicans* is the most common pathogen (36). The serological methods commonly used include agar gel diffusion (AGD), counterimmunoelectrophoresis (CEP), whole cell agglutination (AGGL), latex agglutination (LAT), indirect fluorescent antibody, and complement fixation. Of these, the AGGL test, AGD test, and CEP test are the most frequently employed (19, 22).

A new method, based on the inhibition of clumping factor, has not been fully evaluated extensively to determine its potential value (20).

One of the most common serological tests for systemic candidiasis is the AGGL test developed by Hasenclever and Mitchell (12). A titer of 1:80 or greater is generally regarded as positive, but significance can be attached to a rising titer. This test detects antibody primarily to the mannan component of the candida cell wall. Agglutinating antibody to the mannan components has also been demonstrated in patients with impaired bladder function (9) or with mycotic vulvovaginitis (27). These findings may explain the positive results obtained in patients without systemic candidiasis. Other investigators (1, 7) found a good correlation between infection and agglutination titers.

Taschjian and her associates were among the first to use the precipitin test for the diagnosis of systemic candidiasis. They published extensive evidence that supported this approach for the specific diagnosis of systemic candidiasis (29, 30, 32–35). The antigens commonly used to detect candida precipitins are derived from the cytoplasm of sonically or mechanically disrupted candida cells (10, 16, 22, 29). Commercial culture filtrates have also been used (8, 15, 36). It has been claimed that antibodies to the cytoplasmic antigens occur only in systemic disease, whereas antibodies in the normal population were the result of colonization and were directed to the mannan in the cell wall (29, 35). There has been considerable controversy on this point, and conflicting reports have been published (4–6, 11, 15, 19, 23, 24).

Resolution of this controversy is complicated by the contamination of most cytoplasmic preparations with mannan and the presence of mannan antibody in the sera of patients with systemic candida infections (5, 36). Precipitating antibody to mannan has been found in commercial gamma globulin preparations (6, 15). The extreme variability of antigen preparations and gel diffusion methods obviously also contributes to the lack of uniform results reported from different laboratories (10, 22, 23). In addition, it is generally not appreciated that antigen concentration must be adjusted in the AGD and CEP tests to obtain maximum specificity without losing sensitivity (10, 22).

For these reasons, a study was planned in which six laboratories would perform an AGGL, an LAT, and an AGD test on identical sets of coded sera and would use identical antigen preparations. The objective was to determine which of the three procedures was the most accurate, specific, and reliable. In addition, each of the six laboratories examined the same sera with a test of its choice.

MATERIALS AND METHODS

Selection of sera. One hundred sera were submitted to each of six laboratories as four separate, blindly coded sets. Three sets were tested by each participant in three procedures, and the fourth set was tested with each investigator's "procedure of choice." Table 1 presents information on the 100 sera. The candida-involved group consisted of 53 specimens including sera from 33 proven, 14 presumptive, and 6 probable cases of systemic candidiasis. The negative controls consisted of 41 healthy individuals and 6 sera from patients having either nonmycotic diseases or fungal diseases other than candidiasis.

Common serological tests. The three tests performed by all six laboratories included an AGD, an LAT, and a tube agglutination procedure. Individually coded sets of sera, diagnostic reagents and materials, and detailed instructions were provided for each of the three tests.

(i) AGD test (AGD-1). For this test an experimental kit prepared by Schering Corp., Bloomfield, N.J., was used. The kit consisted of 50-mm dishes of buffered (sodium barbitol) agarose, 1.9 mm in depth, with eight precut wells. The antigen was a homogenate prepared in a homogenizer (Braun MSK) of a culture of *C. albicans* serotype A grown for 48 h at 28°C in proteose peptone broth. The supernatant of the homogenate was filter-sterilized and standardized by testing dilutions of the antigen against a panel of reference sera.

In the test, 20-μl samples of four sera were introduced into four wells (4 mm OD), and 10 μl of the antigen was introduced into each of the four wells (2.7 mm OD), which were spaced 3.0 mm from the sera wells. The plates were incubated at room temperature and read at 24 and 48 h. The presence of one or more precipitin lines between antigen and serum wells was interpreted as a positive reaction. All negative sera were retested with the same procedure before being reported as negative. Positive and negative controls were included in all determinations.

(ii) LAT. Reagents were provided by the Mycology Division of the Center for Disease Control, Atlanta, Ga. Latex particles coated with a cytoplasmic extract of *C. albicans* as described by Stickle et al. (28) were used.

Sera diluted 1:4 were inactivated by heating them at 56°C for 30 min and were then serially diluted

TABLE 1. *Clinical sources and description of the 100 test sera*

Category	No. of:		Remarks
	Sera	Patients	
Proven candidiasis[a]	33[b]	18	Included six cases of *Candida* endocarditis, seven each of disseminated (multiorgan) disease including renal infections, one of intestinal disease, and four other *Candida* infections (panophthalmitis, arthritis, meningitis, and a hip lesion)
Presumptive candidiasis[c]	14[b]	9	Included eight patients with abdominal wounds repeatedly culture positive for *Candida* and one patient with candidal sternal abscesses
Probable candidiasis	6[b]	6	Included patients with catheterized urine cultures repeatedly positive for *Candida*; with cutaneous mucocutaneous *Candida* lesions
Other disease states	6	6	Included six patients with cryptococcal meningitis, *Torulopsis glabrata*, systemic aspergillosis (a pooled specimen), acute myelogenous leukemia, infectious mononucleosis, and systemic lupus erythematosis
Normal	41	41	Nonhospitalized, healthy individuals (controls)

[a] Proven by cultures taken at surgery by pathological sections taken at surgery or at autopsy, or by biopsy.
[b] Most sera had been tested serologically before this study and most were positive.
[c] Established on the basis of multiple positive wound and urine cultures.

(microtiter method) to provide dilutions of 1:4 through 1:128. Agglutination slides were used for testing. Positive and negative control sera were incorporated in each test run. Agglutination reactions equal to or greater than that obtained with the 2+ positive control were interpreted as positive. All sera negative at the 1:4 and 1:8 dilutions were retested to confirm these results. A titer of 1:8 or greater was considered positive.

(iii) **AGGL-1.** A whole cell, *C. albicans* antigen, serotype A, prepared by H. Hasenclever, National Institutes of Health, Bethesda, Md., was used in this test as described by Hasenclever and Mitchell (12). Only one test was performed on each sample. Sera were serially diluted in saline from 1:20 to 1:2,560 in 0.25-ml volumes. To each tube of diluted serum was added 0.25 ml of the whole cell antigen suspension; the tubes were then sealed with Parafilm and incubated in a water bath for 3 h at 37°C. Positive and negative serum controls were included in each test run. The tubes were reincubated at 2 to 8°C for 18 to 24 h. The titer of a positive serum was the highest dilution that produced a visible agglutination. A specimen was regarded as positive if it had a titer of 1:80 or greater.

Individual serological tests. Each of the six participating laboratories selected one additional test, which it performed on the 100 sera. The procedures used included two modifications of an AGD procedure, three forms of CEP, and an AGGL test with several different *Candida* antigens.

Specific details regarding these tests are as follows.

(i) **Microimmunodiffusion test (AGD-2).** This test was conducted with a homogenate of *C. albicans* serotype A in a microsystem as described by Stickle et al. (28). A positive serum was defined as any serum that produced a precipitin line. Positive control sera that produced A, B, and C precipitin lines were also run.

(ii) **Macroimmunodiffusion test (AGD-3).** Differences between this test and the preceding one were the preparation of the cell-free extract antigen, dilution of the antigen, and the use of large plates. This procedure was described by Kozinn et al. (17).

(iii) **CEP.** Three laboratories performed modifications of this test.

CEP-1. The antigen was the same as that used in the AGD-1 test, except that it was used at a 1:10 rather than at a 1:15 dilution. For this test, 100-mm-square petri dishes were filled with 18 ml of buffered 1.0% agarose, pH 8.6 [5.7 g of sodium barbital, 0.73 g of tris(hydroxymethyl)aminomethane, 0.10 g of thimerosal (dry powder), and 10 g of agarose per liter]. Wells were 3.2 mm in diameter, 2.5 mm deep, and spaced 2.5 mm apart. Twenty microliters of each serum sample was introduced into the wells nearest the anode, and 20 μl of antigen was introduced into the wells nearest the cathode. The plates were subjected to electrophoresis in tris(hydroxymethyl)aminomethane/glycine buffer, pH 8.6, for 2 h and then were examined. The appearance of a precipitin line was considered as evidence of a positive reaction. Appropriate positive and negative controls were included in each determination.

CEP-2. Two antigens were used in this procedure. The first was protein antigen that had been prepared from *C. albicans* serotype A grown in submerged culture for 48 h and disintegrated in a Dyno-Mill for 30 min. This antigen was dialyzed against

distilled water and lyophilized; it was tested at reconstituted concentrations (protein) of 5, 0.5, and 0.05 mg/ml. The second antigen was a purified mannan preparation; it was tested at concentrations of 500, 50 and 0.5 μg/ml. Tests were performed in agarose layers 1.4 mm thick prepared on a slide (40 by 70 mm). The wells were 4 mm in diameter and 3 mm apart; 20-μl amounts of antigen or serum were introduced into the wells with sera on the anode side and antigen on the cathode side. Electrophoresis was performed for 90 min in barbital buffer, pH 8.2. After electrophoresis, the slides were examined, washed, and stained with Coomassie brilliant blue. The presence of one or more precipitin bands was considered positive. Sheep anti-*C. albicans* serum was used as a positive control in all determinations.

CEP-3. A commercial preparation of *C. albicans* antigen (Hollister-Stier Laboratory) was used as described by Remington et al. (25).

AGGL. A second AGGL test, AGGL-2, was performed with three separate antigens, *C. albicans* serotype A, *C. albicans* serotype B, and *Candida parapsilosis*. Broth cultures were grown at 30°C for 48 h; then they were harvested, washed by centrifugation, adjusted to contain 1.4×10^7 cells per ml, and heat-killed (50°C for 2 h). Patients' sera were titrated from 1:20 to 1:5,120 in triplicate in 0.25-ml volumes; 0.25 ml of antigen was then added to each dilution and incubated for 3 h at 37°C. Results were determined, and the tubes were then reincubated at 3 to 8°C for 18 h, after which they were again examined. Results were reported in terms of the highest dilution of serum that produced visible agglutination. Positive and negative controls were included in each determination.

Reporting of results. Results from each of the participating laboratories were reported to an independent test moderator. Evaluation of the results and clinical data regarding the specimens were withheld from the participants until all data were received by the moderator, who then broke the code and analyzed the data.

RESULTS

The results obtained with the AGD-1 test from each laboratory are shown in Table 2. In the candida-involved group, the positive results by laboratory ranged from 58.5 to 94.3%. In the negative control group, positive results ranged from 2.1 to 6.4%. The AGD-1 test in five of the six laboratories had 85% or greater positives except for one laboratory. The overall positive average for the candida-involved group would increase from 85.1 to 90.6% if results from this laboratory were eliminated.

Table 3 shows the results with the LAT. The positive results in the candida-involved group ranged from 83 to 98.1% and, in the negative controls, from 8.5 to 36.2%. The results by labo-

TABLE 2. *Results of the AGD-1 test from six laboratories*

Serum category (no. of samples)	Laboratory					
	1	2	3	4	5	6
Proven (33)	31[a]	30	28	17	31	26
Presumptive (14)	14	14	14	10	14	13
Probable (6)	5	4	5	4	5	5
Totals (53)	50	48	47	31	50	45
Percent positive	94.3	90.6	88.7	58.5	94.3	84.9
Negative controls (47)	2[a]	3	3	1	3	2
Percent positive	4.3	6.4	6.4	2.1	6.4	4.3

[a] Number of sera giving positive results.

TABLE 3. *Results of the LAT from six laboratories*

Serum category (no. of samples)	Laboratory					
	1	2	3	4	5	6
Proven (33)	30[a]	31	29	33	32	28
Presumptive (14)	13	12	11	10	14	12
Probable (6)	4	5	4	4	6	5
Totals (53)	47	48	44	47	52	45
Percent positive	88.7	90.6	83.0	88.7	98.1	84.9
Negative controls (47)	8[a]	10	4	5	17	5
Percent positive	17.0	21.3	8.5	10.6	36.2	10.6

[a] Number of sera giving positive results.

ratory were fairly consistent in the candida-involved group, but the variation in the control group makes it difficult to assess the value of this test.

In Table 4 are shown the results with the AGGL-1 test. In the candida-involved group, positive results ranged from 45.3 to 88.7%. The results were consistently lower than the other two tests, except for one laboratory, which obtained 88.7% positive results. In the negative control group, the positive results ranged from 0 to 44.7%. This considerable variation makes evaluation difficult, although low sensitivity is apparent.

The results of the individual "test of choice" from each of the six participating laboratories are shown in Table 5. In the candida-involved category of sera, the AGGL-2 test produced the second highest number of positives (94.3%) but also gave the second highest number of false positives (19.1%). The AGD-3 procedure produced the fewest positives (83%) in the candida-involved category and a fairly high number of false positives (10.6%). The CEP-1 procedure produced no false positives but only 88.7% true positives. The AGD-2, CEP-2, and CEP-3 procedures all produced greater than 90% true positive results, but only the CEP-3 procedure had a low number (2.1%) of false positives.

Although not shown in the data, it should be pointed out that there were three sera from proven cases that were reported as negative in five or more of the nine tests. One of these was from a patient with candida panophthalmitis, one was from a patient with multiple candidal abscesses of the stomach and colon, and one serum came from a patient with abscesses of the kidney, stomach, and esophagus. It should also be noted that one of the sera from the normal control population gave positive reactions by most of the nine tests.

The sensitivity and specificity of each of the nine tests are shown in Table 6. Of the three common tests, AGD-1 was the most specific (93.6%) and had fairly high sensitivity (85.1%). The LAT was the most sensitive (89%) of the three common tests but was least specific (82.6%). The AGGL-1 test was the least sensitive of all nine tests. The most sensitive of the individual tests was CEP-2 (at 96.2%), but it also was the least specific (76.6%). Among the individual tests, the CEP-3 gave the best performance, with CEP-1 a close second.

The reproducibility, by laboratory, of the three common tests is presented in Tables 7 to 9. It is apparent from these results that the AGD-1 test gave the most consistent results, and the AGGL-1 was the least reproducible.

TABLE 4. *Results of the AGGL-1 test from six laboratories*

Serum category (no. of samples)	Laboratory					
	1	2	3	4	5	6
Proven (33)	30[a]	14	22	19	24	18
Presumptive (14)	13	6	8	7	10	9
Probable (6)	4	4	6	3	3	3
Totals (53)	47	24	36	29	37	30
Percent positive	88.7	45.3	67.9	54.7	69.8	56.6
Negative controls (47)	5[a]	0	21	6	1	2
Percent positive	10.6	0	44.7	12.8	2.1	4.3

[a] Number of sera giving positive results.

TABLE 5. *Results of individual tests*

Serum category (no. of samples)	AGGL-2	AGD-2	AGD-3	CEP-1	CEP-2	CEP-3
Proven (33)	30[a]	30	29	31	31	31
Presumptive (14)	14	14	12	11	14	13
Probable (6)	6	5	3	5	6	5
Totals (53)	50	49	44	47	51	49
Percent positive	94.3	92.5	83.0	88.7	96.2	92.5
Negative controls (47)	9[a]	5	5	0	11	1
Percent positive	19.1	10.6	10.6	0	23.4	2.1

[a] Number of sera giving positive results.

TABLE 6. *Sensitivity and specificity of each test*

Criterion	Common tests			Individual tests					
	AGD-1	LAT	AGGL-1	AGGL-2	AGD-2	AGD-3	CEP-1	CEP-2	CEP-3
Sensitivity[a]	85.1[b]	89.0	63.8	94.3	92.5	83.0	88.7	96.2	92.5
Specificity[c]	93.6	82.6	87.6	80.9	89.4	89.4	100	76.6	97.9

[a] Percent positive, combined results with proven (33), presumptive (14), and probable (6) sera.
[b] Numbers indicate average results from six laboratories.
[c] Percent negative with 47 control sera.

TABLE 7. *Reproducibility of the AGD-1 test by laboratory*

No. of laboratories reporting positive	Category of sera tested			
	Proven	Presumptive	Probable	Controls
0	2	0	1	43
1	0	0	0	1
2	1	0	0	1
3	1	0	0	0
4	4	1	1	0
5	8	3	0	1
6	17	10	4	1

TABLE 8. *Reproducibility of the LAT by laboratory*

No. of laboratories reporting positive	Category of sera tested			
	Proven	Presumptive	Probable	Controls
0	1	0	0	29
1	0	1	0	6
2	0	1	1	4
3	3	0	1	2
4	2	1	0	3
5	7	1	1	1
6	20	10	3	2

TABLE 9. *Reproducibility of the AGGL test by laboratory*

No. of laboratories reporting positive	Category of sera tested			
	Proven	Presumptive	Probable	Controls
0	1	0	0	22
1	5	1	1	19
2	3	4	1	2
3	4	1	0	4
4	4	2	2	0
5	8	2	0	0
6	8	4	2	0

This is also apparent from Tables 2 to 4.

The investigators were asked to rate each of these tests for ease of interpretation. The AGD-1 was rated easiest to interpret, the LAT was second, and the AGGL-1 was rated as the most difficult. This obviously helps to explain the difference in reproducibility. The personnel of only one of the six participating laboratories were inexperienced with AGD procedures, and this laboratory obtained significantly fewer positive results with the AGD-1 test than the others.

DISCUSSION

This was a laboratory study designed to compare the various serological tests for systemic candidiasis reported in the literature. The sera in the candida-involved group were from patients with well-documented clinical conditions. The "other disease states" group consisted of only six patients, and only three of these had mycoses other than candida. Thus, the specificity of the tests was challenged only with respect to normal individuals.

There were considerable differences among the three AGD tests used in this study. This procedure has been reported by some authors as both sensitive and specific (30, 36). The AGD-1 test gave only five false negatives and two false positives, thus demonstrating good sensitivity and specificity. The AGD-2 gave only four false-negative reactions, but five false-positive reactions. It, therefore, had greater sensitivity but lower specificity than the AGD-1. The AGD-3 gave nine false-negative and five false-positive reactions. It was less sensitive than the other two and had a specificity identical to the AGD-2. Since there were no major difficulties in interpreting the AGD-1 test except in one laboratory, it seems that the differences in these tests lay entirely in the reagents used. The argument that frozen sera lose their reactivity (30, 35) in AGD tests is not valid for explaining the variability of results, since many of the positive sera in the present study were stored for several years in the frozen state. Although cell-free extracts of *C. albicans* were used in all three AGD tests, variation was probably due to different environmental conditions for growth, methods of disruption, strain-to-strain variation in antigenic composition, dilution factors, and different test conditions.

The LAT procedure is similar to both the AGGL and the AGD procedures. It is similar to the AGGL test in that an agglutination reaction is produced and a titer is determined. It is similar to the AGD tests in that the latex particles are coated with a cell-free extract of *C. albicans*. The test is, however, more rapid than the other two procedures. The latex test had an 85% sensitivity and an 87% specificity. The test, however, offers the opportunity to quantitate antibody responses and when used concomitantly with an AGD procedure provides diagnostic and prognostic data.

AGGL tests have been reported (35) to give many false-positive reactions. The AGGL-1 in this study showed the reverse, with very few false positives but a significant number of false negatives. This was, in part, due to difficulties in interpreting the results, as illustrated by the range of 24 to 47 positive reports from the various laboratories out of a possible 53 in the candida-involved group. Results with the AGGL-2, however, did agree with those reported in the literature. There were only three false negatives out of 53 in the candida-involved group (however, there were nine false positives out of 47). The increased number of positives could have been due to the use of three different cells with different antigenic compositions. Therefore, this very sensitive test could be used for screening purposes. Sera found positive with this test could then be tested in a more specific test.

CEP tests for the detection of anti-candida antibodies have been reported to be very sensitive (15, 22, 25). There were variations in the results with the three CEP procedures used in our study. The CEP-2 test that used a cell wall mannan antigen and a protein antigen was extremely sensitive; however, it gave many false-positive reactions. Although not shown in Table 5, there was no appreciable difference between the protein antigen and the mannan antigen results in either the candida-involved group or the negative control group with this procedure. The oversensitivity of this test may have been due to a low concentration of mannan in the protein antigen, which would detect very low levels of antibodies directed against the cell wall (personal communication with the investigator). The CEP-1 test gave no false-positive reactions, but did give five false-negative reactions. The CEP-3 had both few false negatives and few false positives. The differences in the results with these three similar procedures were due mainly to variations in the antigenic composition of the cell-free extracts and, secondarily, to the conditions of electrophoresis.

Since this study was not designed to give data concerning a clinical evaluation of these tests, no conclusion can be drawn on how many false positives might occur with these tests in a population of patients at high risk to develop deep or systemic candidiasis. Therefore, each test should be run against a standard panel of sera from patients with known deep or systemic candidiasis, colonization due to candida, superficial candidiasis, and no candidiasis. The test can then be adjusted to yield maximum number of true-positive reactions with minimal false-positive reactions. This study clearly demonstrated that if these procedures are improved by determining the optimal conditions for their performances and by the use of standardized reagents, a laboratory test can be established that would be of great value in the diagnosis of systemic candidiasis.

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LITERATURE CITED

1. **Andersen, P. L., and A. Stenderup.** 1974. *Candida albicans* antibodies in candidiasis. Scand. J. Infect. Dis. 6:69–73.
2. **Axelsen, N. H.** 1971. Antigen-antibody crossed electrophoresis (Laurell) applied to the study of the antigenic structure of *Candida albicans*. Infect. Immun. 4:525–527.
3. **Bernhardt, H. E., J. C. Orlando, J. R. Benfield, F. M. Hirose, and R. Y. Foos.** 1972. Disseminated candidiasis in surgical patients. Surg. Gynecol. Obstet. 134:819–825.
4. **Bläker, F., K. Fischer, and H. H. Hellwege.** 1973. Significance of humoral antibodies against *Candida albicans* in the diagnosis of candida infections. Dtsch. Med. Wochenschr. 98:194–201.
5. **Buckley, H. R., and E. W. Lapa.** 1969. The value of serological tests in the diagnosis of candidiasis. Antonie van Leeuwenhoek J. Microbiol. Serol. 35 (Suppl.):E19–E20.
6. **Chew, W. H., and T. L. Theus.** 1967. Candida precipitins. J. Immunol. 98:220–224.
7. **Comaish, J. S., B. Gibson, and C. A. Green.** 1967. Candidiasis—serology and diagnosis. J. Invest. Dermatol. 30:139–142.
8. **Dolan, C. T., and R. P. Stried.** 1973. Serologic diagnosis of yeast infections. Am. J. Clin. Pathol. 59:49–55.
9. **Everall, P. H., C. A. Morris, and D. F. Morris.** 1974. Antibodies to *Candida albicans* in hospital patients with and without spinal injury and in normal men and women. J. Clin. Pathol. 27:722–728.
10. **Faux, J. A., V. C. Stanley, H. R. Buckley, and B. M. Partridge.** 1975. A comparison of different extracts of *Candida albicans* in agar gel double diffusion techniques. J. Immunol. Methods 6:235–247.
11. **Goldstein, E., and P. D. Hoeprich.** 1972. Problems in the diagnosis and treatment of systemic candidiasis. J. Infect. Dis. 125:190–193.
12. **Hasenclever, H. F., and W. O. Mitchell.** 1961. Observation of two antigenic groups in *Candida albicans*. J. Bacteriol. 82:570–573.
13. **Hasenclever, H. F., and W. O. Mitchell.** 1963. Antigenic studies of candida. IV. The relationship of the

antigenic groups of *Candida albicans* to their isolation from various clinical specimens. Sabouraudia 2:201-204.
14. Hasenclever, H. F., W. O. Mitchell, and J. Loewe. 1961. Antigenic studies of candida. II. Antigenic relation of *Candida albicans* group A and group B to *Candida stellatoidia* and *Candida tropicalis*. J. Bacteriol. 82:574-577.
15. Hellwege, H. H., K. Fischer, and F. Blaker. 1972. Diagnostic value of candida precipitins. Lancet ii:386.
16. Kemp, G., and M. Soltorovsky. 1964. Localization of antigens in mechanically disrupted cells of certain species of the genera *Candida* and *Torulopsis*. J. Immunol. 93:305-314.
17. Kozinn, P. J., R. S. Galen, C. L. Taschdjian, P. K. Goldberg, W. P. Protzman, and M. A. Kozinn. 1976. The precipitin test in systemic candidiasis. J. Am. Med. Assoc. 235:628-629.
18. Kozinn, P. T., H. F. Hasenclever, C. L. Taschdjiian, D. W. Mackenzie, W. Protzman, and M. S. Seelig. 1972. Problems in the diagnosis and treatment of systemic candidiasis. J. Infect. Dis. 126:548-550.
19. Lehner, T., H. R. Buckley, and I. G. Murray. 1972. The relationship between fluorescent, agglutinating and precipitating antibodies to *Candida albicans* and their immunoglobulin classes. J. Clin. Pathol. 25:344-348.
20. Louria, D. B., J. K. Smith, R. G. Brayton, and M. Buse. 1972. Anti-*Candida* factors in serum and their inhibitors. I. Clinical and laboratory observations. J. Infect. Dis. 125:102-114.
21. Murray, I. G., H. R. Buckley, and G. C. Turner. 1969. Serological evidence of candida infection after open heart surgery. J. Med. Microbiol. 2:463-469.
22. Odds, F. C., E. G. V. Evans, and K. T. Holland. 1975. Detection of candida precipitins. A comparison of double diffusion and counter immunoelectrophoresis. J. Immunol. Methods 7:211-218.
23. Pepys, J., J. A. Faux, D. S. McGarthy, J. Longbottom, and F. E. Hargreave. 1968. *Candida albicans* precipitins in respiratory disease in man. J. Allergy 41:305-318.
24. Preisler, H. D., H. F. Hasenclever, A. A. Levitan, and E. S. Henderson. 1969. Serologic diagnosis of disseminated candidiasis in patients with acute leukemia. Ann. Intern. Med. 70:19-30.
25. Remington, J. S., J. D. Gaines, and M. A. Gilmer. 1972. Demonstration of candida precipitins in human sera by counterimmunoelectrophoresis. Lancet i:413.
26. Rosner, F., F. D. Gabriel, C. L. Taschdjiian, M. B. Cuesta, and P. J. Kozinn. 1971. Serologic diagnosis of systemic candidiasis in patients with acute leukemia. Am. J. Med. 51:54-62.
27. Stanley, V. C., R. Hurley, and C. J. Carrol. 1972. Distribution and significance of candida precipitins in sera from pregnant women. J. Med. Microbiol. 5:313-320.
28. Stickle, D., L. Kaufman, S. O. Blumer, and D. W. McLaughlin. 1972. Comparison of a newly developed latex agglutination test and immunodiffusion test in the diagnosis of systemic candidiasis. Appl. Microbiol. 23:490-499.
29. Taschdjian, C. L., G. B. Dobkin, L. Caroline, and P. J. Kozinn. 1964. Immune studies relating to candidiasis. II. Experimental and preliminary clinical studies on antibody formation in systemic candidiasis. Sabouraudia 3:129-139.
30. Taschdjian, C. L., P. J. Kozinn, M. B. Cuesta, and E. F. Toni. 1972. Serodiagnosis of candidal infections. Am. J. Clin. Pathol. 57:195-205.
31. Taschdjian, C. L., P. J. Kozinn, and E. F. Toni. 1970. Opportunistic yeast infections, with special reference to candidiasis. Ann. N.Y. Acad. Sci. 174:602-622.
32. Taschdjian, C. L., P. J. Kozinn, A. Okas, L. Caroline, and M. A. Halle. 1967. Serodiagnosis of systemic candidiasis. J. Infect. Dis. 117:180-187.
33. Taschdjian, C. L., P. J. Kozinn, and L. Caroline. 1964. Immune studies in candidiasis. III. Precipitating antibodies in systemic candidiasis. Sabouraudia 3:312-320.
34. Taschdjian, C. L., P. J. Kozinn, H. Fink, M. B. Cuesta, L. Caroline, and A. B. Kantrowitz. 1969-70. Post mortem studies of systemic candidiasis. I. Diagnostic validity of precipitin reaction and probable origin of sensitization of cytoplasmic candidal antigens. Sabouraudia 7:110-117.
35. Taschdjian, C. L., M. S. Seelig, and P. J. Kozinn. 1973. Serologic diagnosis of candidal infections. CRC Crit. Rev. Clin. Lab. Sci. 4:19-59.
36. Taschdjian, C. L., M. B. Cuesta, P. J. Kozinn, and L. Caroline. 1969. A modified antigen for serologic diagnosis of systemic candidiasis. Am. J. Clin. Pathol. 52:468-472.
37. Toala, P., S. A. Schroeder, K. A. Daly, and M. Finland. 1970. Candida at Boston City Hospital. Arch. Intern. Med. 126:983-989.