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Nonvalue of Antigen Detection Immunoassays for Diagnosis of Candidemia

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We evaluated the Cand-Tec (Ramco Laboratories Inc., Houston, Tex.) and LA-*Candida* antigen detection system (Immuno-Mycologics Inc., Norman, Okla.) tests as possible rapid alternatives to blood cultures for the identification of patients with candidemia. Tests were performed on sera from (i) 33 patients with candidemia, (ii) 82 patients with fever and risk factors for invasive candidiasis, and (iii) 13 healthy controls. A total of 21 patients had no evidence of invasive candidiasis, as determined by clinical course, blood culture, and/or autopsy; results for 61 patients were indeterminate regarding the presence of invasive candidiasis, or else the patients had invasive candidiasis with organ involvement. By using a threshold positive Cand-Tec titer of ≥1:4, the sensitivity in candidemic patients was 49%; the specificity was 43% (patients with true-negative results had neither candidemia nor other evidence of invasive candidiasis). Coexistent disseminated candidiasis in some candidemic patients may have accounted for some positive Cand-Tec tests and possible overestimation of the sensitivity of the test for candidemia. Cand-Tec test results were negative for healthy controls. All test results obtained by the LA-*Candida* antigen detection system assay were negative. Our findings indicate that neither of these assays reliably identifies patients with candidemia.

Lysis-centrifugation is considered the "gold standard" test for the diagnosis of candidemia (6, 19, 21). Regardless of the blood culture technique used, it may take up to several days to establish the diagnosis of candidemia.

The toxicity of intravenous amphotericin B has limited the empiric use of the drug. The only widely accepted indication for empiric amphotericin B is febrile neutropenia, which remains unresponsive to broad-spectrum antibacterial therapy (26). Invasive candidiasis also involves surgical patients (7, 23), those with long-term indwelling vascular catheters (18), burn patients (28), low-birth-weight neonates (1), and nonneutropenic immunosuppressed patients (16). The reluctance to use empiric systemic antifungal therapy underscores the importance of developing rapid and accurate diagnostic methods for candidemia.

Several reports have detailed the accuracy of commercially available immunoassays for *Candida* antigen in the diagnosis of invasive candidiasis (2, 8–10, 14, 15, 17, 20, 22, 24, 25, 27, 30, 31). Results of initial studies have indicated that both the sensitivity and the specificity of the Cand-Tec assay are sufficiently high to warrant routine use of this assay in the evaluation of high-risk patients (8, 10, 15, 17). However, other investigators had less favorable experiences with this immunoassay (2, 14, 22, 24). Studies that have been performed to date have included patients with various forms of invasive candidiasis, but they have not specifically addressed the application of antigen detection methods to subgroups of patients with candidemia. We evaluated two latex agglutination immunoassays for *Candida* antigen in the diagnosis of candidemia.

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MATERIALS AND METHODS

Definitions. Candidemia was defined as one or more blood cultures positive for *Candida* spp. and an oral temperature of ≥38°C. Disseminated candidiasis was defined as histologic (with or without microbiologic) evidence of tissue invasion by organisms morphologically consistent with *Candida* spp. in two or more viscera. Focal invasive candidiasis referred to histologic (with or without microbiologic) evidence of tissue invasion of one organ (e.g., liver) or a clinical syndrome strongly suggestive of invasive candidal infection involving one organ (e.g., endophthalmitis). Candidiasis with organ involvement referred collectively to focal invasive or disseminated candidiasis. Invasive candidiasis was used as a term that encompassed all of the forms of candidal infection mentioned above but excluded the various forms of mucocutaneous candidiasis (e.g., thrush, esophagitis, and vaginitis).

Patients. Hospitalized patients were prospectively enrolled in the study if they had (i) one or more risk factors for invasive candidiasis; (ii) fever, which was defined as an oral temperature of ≥38°C; and (iii) lack of response to antibacterial therapy that was administered for ≥4 days. The factors that put patients at risk for candidiasis and that were used as criteria for patient enrollment in the study included (i) neutropenia ($<0.5 \times 10^9$/liter), (ii) use of immunosuppressive therapy, (iii) use of total parenteral nutrition, (iv) broad-spectrum antibiotic administration, (v) presence of long-term intravascular catheters, (vi) gastrointestinal surgery during the same hospitalization, and (vii) the presence of burn wounds. Routine blood cultures (both lysis-centrifugation and BACTEC 660 bottles [Johnston Laboratories, Inc., Towson, Md.]) were used for the investigation of candidiasis. Sera collected on the same day that specimens

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for blood cultures were obtained were also used for the immunoassays.

Sera were also selected from other patient specimens that were refrigerated (1 to 8°C) and held in the blood chemistry laboratory for 13 days or less. Sera stored for longer periods were kept at −70°C. This latter group consisted of sera from patients with either documented candidemia or febrile illness associated with risk factors for invasive candidiasis and either bacteremia or sterile blood cultures.

Patients were divided into group A, B, or C based on the laboratory evidence of *Candida* infection. Group A patients were candidemic and were subdivided into those with one and those with more than one (drawn at least 30 min apart) blood cultures positive for *Candida* sp. Patients were categorized as negative controls (group B), provided that a *Candida* sp. was not isolated from blood or other normally sterile body fluids and that there was no other evidence of invasive candidiasis. Assignment to group B was considered appropriate if a fatal outcome was not associated with evidence of invasive candidiasis at the time of autopsy or if recovery from a febrile illness occurred in the absence of any systemic antifungal therapy or evidence of invasive candidiasis during hospitalization or at a minimum follow-up period of 3 months. Group C consisted of patients with focal invasive or disseminated candidiasis and was collectively referred to as patients with candidiasis with organ involvement. Group D was made up of healthy volunteers.

Results for the remaining patients were indeterminate with respect to the presence of invasive candidiasis. Such patients had blood cultures negative for *Candida* spp., and the possibility of invasive candidiasis could not be established or excluded. Patients for whom the results were indeterminate included those who died during the hospitalization and did not undergo autopsy examination or those who received empiric amphotericin B therapy and survived but in whom invasive candidiasis was not documented.

Sera from eight candidemic patients were not available for antigen detection on the same day that at least one positive blood culture was obtained. These patients were excluded from the final analysis. One patient with a brain abscess caused by *Pseudallescheria boydii* (classified as a negative control patient versus a patient with candidiasis with organ involvement) was excluded from the final analysis because of the uncertain significance of a postmortem brain biopsy culture which yielded both *Candida* sp. (in one of four cultures) and *P. boydii* (in four of four cultures), with only histologic evidence of *P. boydii* infection.

Patients were enrolled in the study according to a protocol approved by the University of British Columbia Ethics Committee for studies involving human trials. The study was performed between May 1988 and November 1989 at three university-affiliated hospitals: Vancouver General, St. Paul's, and the University Hospital. Demographic and clinical data were recorded for all patients. Clinical-pathologic correlation was obtained when pathology results were available. Individual serum samples were assigned a study number at the time of patient enrollment, and all immunoassays were performed by an observer who was blinded to the clinical and microbiologic data relevant to the case.

Latex agglutination immunoassays. (i) Cand-Tec assay. The Cand-Tec assay (Ramco Laboratories Inc., Houston, Tex.) was performed by the methods indicated in the product insert, as revised in March 1989. Twenty microliters of a serum sample, which was diluted 1:2 with the diluent, was mixed with 20 μl of *Candida*-sensitive latex (latex beads coated with anti-*Candida* antibody) on a spot on a reusable glass slide provided with the assay kit. Serum dilutions of 1:2, 1:4, 1:8, etc., were tested until a negative result was obtained. The slide was rotated horizontally at 140 rpm for 10 min, and samples were observed (without magnification) for agglutination of the latex beads compared with agglutination of the positive and negative controls provided with the assay kit. Sera which had titers of 1:4 or greater were also tested for rheumatoid factor.

(ii) LA-*Candida* antigen detection system. The LA-*Candida* antigen detection system (LA-CADS; Immuno-Mycologics Inc., Norman, Okla.) assay was performed by the methods indicated in the product insert. In order to dissociate mannan-containing immune complexes, the serum samples (0.3 ml each) were digested at 56°C for 30 min in the proteolytic enzyme (0.05 ml per sample) provided with the assay kit. One drop of the enzyme inhibitor was added to each specimen, which was then tested for *Candida* antigen by mixing 2 drops (50 μl) of treated serum with 1 drop (25 μl) of anti-*Candida* globulin reagent (latex beads coated with antibodies) on the test cards provided with the assay kit. The test cards were placed in a moist chamber which was rotated at 160 rpm for 10 min. The presence of latex bead agglutination was read visually without magnification. Positive and negative control tests were performed simultaneously with the reagents provided with the assay kit.

Statistical analysis. The data were analyzed by the chi-square test.

RESULTS

Sera from the following groups of patients were tested: candidemia, 33 patients; negative control patients, 21 patients; candidiasis with organ involvement, 9 patients; and healthy volunteers, 13 subjects. Fifty-two cases were indeterminate with respect to the presence or absence of invasive candidiasis.

Outlines of the various clinical groups and Cand-Tec titers in sera from candidemic patients, negative control patients, and those with focal and disseminated candidiasis with organ involvement are given in Tables 1, 2, and 3, respectively. By using a titer of 1:4 or greater as the cutoff value for a positive result, 49% (95% confidence interval, 32 to 66%) of the candidemic patients had a positive test result by the Cand-Tec assay. By using sera from the negative control patients, the specificity was 43% (95% confidence interval, 22 to 64%). By using a threshold titer of 1:8 or greater for a positive Cand-Tec assay result, the sensitivity fell to 6% and the specificity increased to 62%. If the positive threshold titer was set at 1:2, the sensitivity and specificity were 72 and 29%, respectively. The correlation coefficient between blood culture documentation of candidemia and the Cand-Tec assay (positive titer, of ≥1:4) was −0.68. For sera from a selected high-risk patient population with an estimated candidemia prevalence of 10%, the positive and negative predictive values by the Cand-Tec assay (positive titer, ≥1:4) were 9 and 88%, respectively. Positive titers of 1:4 or greater were noted in sera from 4 (44%) of the patients in group C. The titers in sera from the various clinical groups are presented in Fig. 1.

Sera were available for the rheumatoid factor assays for 37 of the patients who had positive Cand-Tec titers (≥1:4). These included candidemic patients (n = 13), negative control patients (n = 11), patients with candidiasis with organ involvement (n = 3), and patients for whom the results were indeterminate (n = 10). One patient with candidiasis and organ involvement (Table 3, patient 5) was positive for

TABLE 1. Cand-Tec titers[a]

Patient type and patient no.	Age (yr)	Sex	Underlying disease	*Candida* sp. (no. of positive blood cultures)[b]	Titer	Serum creatinine (μmol/liter)	Treatment (dose [mg])	Outcome
Candidemic								
1	66	M	Peritonitis	*C. albicans* (2)	1:4	160	AMB (770)	Survival
2	36	M	Leukemia (ALL)	*C. albicans* (3)	Neg	116	AMB (1,122)	Survival
3	75	M	Myocardial infarct	*C. albicans* (13)	1:4	187	AMB (585)	Death
4	69	M	Burn wounds	*C. albicans* (4)	1:4	183	AMB (50)	Death
5	22	M	BMT (CLL)	*C. tropicalis* (3)	1:4	92	AMB (1,010)	Death
6	63	M	Myelodysplasia	*C. lusitaniae* (6)	1:2	54	AMB (2,236)	Survival
7	69	M	Bowel resection	*C. alibicans* (5)	Neg	118	AMB (500)	Survival
8	28	M	Peritonitis	*C. albicans* (3)	1:4	530	AMB (190)	Death
9	69	M	Cirrhosis	*C. albicans* (3)	1:2	112	AMB (130), 5-FC	Death
10	49	M	Pancreatitis	*C. albicans* (5)	Neg	116	AMB (226), FLU	Survival
11	30	F	Hyperemesis	*C. albicans* (2)	Neg	47	Nil	Survival
12	37	F	Respiratory failure	*C. albicans* (6)	1:8	254	AMB (385)	Death
13	48	F	Peritonitis	*C. albicans* (5)	1:4	60	AMB (70)	Death
14	52	F	BMT (CML)	*C. albicans* (5)	Neg	302	AMB (1,050)	Death
15	62	M	Pancreatitis	*C. albicans* (3)	1:8	999	Nil	Death
16	26	F	BMT (CML)	*C. albicans* (7)	Neg	78	AMB (1,731)	Survival
17	1	M	Short bowel	*C. albicans* (9)	1:2	20	AMB (133), 5-FC	Survival
18	27	M	Leukemia (AML)	*C. albicans* (8), *C. parapsilosis* (4)	1:4	83	AMB (1,500), 5-FC	Survival
19	32	F	BMT (myeloma)	*C. albicans* (2)	1:2	141	AMB (1,500)	Survival
20	49	M	Leukemia (AML)	*C. albicans* (4)	1:4	118	AMB (2,200), 5-FC	Survival
21	52	M	Cardiomyopathy, lymphoma	*C. albicans* (2)	1:2	102	AMB	Survival
Candidemic with only one positive blood culture								
1	80	M	Subphrenic abscess	*C. albicans*	1:4	113	Nil	Death
2	30	M	Bowel resection	*C. albicans*	Neg	83	Nil	Survival
3	62	F	Bronchiectasis	*Candida* sp.	1:4	117	Nil	Survival
4	18	M	Burn wounds (60%)	*C. albicans*	1:4	113	Nil	Death
5	63	M	Multiple trauma	*C. albicans*	1:2	380	Nil	Death
6	47	M	Subphrenic abscess	*C. albicans*	1:4	88	AMB (1,650)	Death
7	68	M	Bowel resection	*C. albicans*	1:4	509	AMB (380)	Death
8	26	F	BMT (CML)	*C. tropicalis*	1:4	78	AMB (1,731)	Survival
9	55	M	Heart transplant	*C. albicans*	Neg	378	AMB (261)	Death
10	34	F	BMT (ALL)	*C. albicans*	1:2	92	AMB (1,016)	Survival
11	68	M	Urinary calculi	*C. albicans*	Neg	60	Nil	Survival
12	42	M	Myelodysplasia	*C. albicans*	1:2	231	AMB	Survival

[a] Abbreviations: M, male; F, female; ALL and CLL, acute and chronic lymphocytic leukemia, respectively; BMT, bone marrow transplant; AML and CML, acute and chronic myelogenous leukemia, respectively; Neg, negative; AMB, amphotericin B; 5-FC, flucytosine; FLU, fluconazole.
[b] Positive blood cultures were separated by at least 0.5 h.

rheumatoid factor; all other patients were negative for this assay.

A total of 106 serum samples from 70 patients were tested by the LA-CADS, including patients with candidemia (n = 11), negative control patients (n = 8), patients with candidiasis with organ involvement (n = 5), and patients for whom the results were indeterminate (n = 41). LA-CADS was also applied to sera from five other patients with candidemia (not group A), from whom sera were collected between 1 and 4 days after blood cultures became positive. None of these samples was positive, despite the presence of functional positive and negative controls as supplied in the assay kit.

Among 111 patients with simultaneous Cand-Tec assay results and serum creatinine levels, 29 and 16% with positive and negative titers, respectively, had creatinine levels greater than 200 μmol/liter ($P > 0.05$).

Five patients with candidemia had isolates in blood other than *Candida albicans*. These were *Candida tropicalis* (n = 2), *Candida lusitaniae* (n = 1), and *Candida* sp. (n = 1); one patient had fungemia with both *C. albicans* and *Candida parapsilosis*. Cand-Tec titers were ≥1:4 for sera from each of these patients except for the patient infected with *C. lusitaniae*.

DISCUSSION

Frequently, patients with candidemia, disseminated candidiasis, or both present with a nonspecific febrile illness (12), often while receiving broad-spectrum antibiotics. A minority of patients have characteristic retinal (18) or skin lesions (12). Patients with candidemia represent a heterogeneous group, including those who may recover without sequelae or subsequently develop focal invasive or disseminated candidiasis or those who already have an established visceral infection at the time of candidemia. Occasional positive blood cultures for candidemia may represent specimen contamination (33, 34). It may be difficult to exclude the coexistence of focal invasive or disseminated candidiasis in candidemic patients, although such complications occur more frequently in neutropenic hosts (4). Candidemia in the absence of a vascular catheter generally indicates the presence of an endovascular infection or disseminated disease. Among those patients with candidemia, it is also difficult to identify those who will proceed to develop metastatic sites of infection.

It has been suggested that candidemia, in the absence of serious immunocompromise (e.g., neutropenia), does not

TABLE 2. Cand-Tec titers in negative controls[a]

Patient no.	Age (yr)	Sex	Underlying disease	Documented infection	PM and FU[b]	MUC/COL[c]	Titer	Serum creatinine (μmol/liter)
1	85	F	Laparotomy, MI	*Pseudomonas* pneumonia	Yes		1:8	137
2	48	M	BMT (myelodysplasia)	Disseminated CMV	Yes	S	1:16	140
3	82	M	Bronchiolitis obliterans	Possible viral pneumonia	Yes	S	1:8	105
4	77	F	Perforated bowel	Peritonitis	Yes	W	1:2	272
5	33	F	Heart-lung transplant	CMV bronchitis	Yes		1:2	94
6	35	M	Burn wounds	*Enterobacter* sepsis	Yes	W	1:4	175
7	78	M	Coronary artery disease	Possible pneumonia	Yes	U	1:16	257
8	60	F	Hepatic necrosis	Disseminated aspergillosis	Yes	Eso[d]	1:16	191
9	46	M	Gastrointestinal bleeding, laparotomy	Staphylococcal bacteremia	Yes	S, U, W, L	Neg	394
10	57	M	Lymphoma	*Pseudomonas* UTI	Yes	U	1:8	52
11	52	F	BMT (myelodysplasia)	None	Yes		Neg	178
12	73	F	Tuberculosis	*Enterobacter* sepsis	Yes		Neg	67
13	33	F	Leukemia (AML)	None	Yes		1:4	333
14	19	F	BMT (ALL)	Oral HSV, vaginal candidiasis	No (20)	Vag/V, L	Neg	51
15	31	F	Leukemia (AML)	Oral HSV	No (9)		Neg	77
16	40	M	Leukemia (AML)	None	No (12)		1:4	73
17	41	F	Intravenous drug abuse	Staphylococcal sepsis	No (12)	U	1:8	98
18	58	M	Aortic valve replacement	*Enterobacter* sternal wound infection	No (3)		1:8	160
19	29	F	Cesarean delivery, respiratory failure	*Candida* UTI[e], *Pseudomonas* UTI	No (10)	UTI/F	1:4	58
20	69	M	Bowel perforation	*Klebsiella* sepsis	No (13)	S	Neg	56
21	32	M	Systemic lupus	Cryptococcal meningitis	No (5)		1:2	85

[a] Abbreviations: F, female; M, male; MI, myocardial infarction; BMT, bone marrow transplantation; CMV, cytomegalovirus infection; UTI, urinary tract infection; AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; HSV, herpes simplex virus infection.

[b] PM, Postmortem was (yes) or was not (no) performed; numbers in parentheses are duration of follow-up (FU) (in months) in survivors.

[c] MUC-COL, Mucosal candidiasis/sites colonized with *Candida* sp. Mucosal candidiasis involved the esophagus (Eso), vagina (Vag), or urinary tract (UTI). Colonized sites included sputum (S), wound (W), urine (U), vagina (V), feces (F), and vascular line (L).

[d] eso, Diagnosis based on characteristic endoscopic appearance only.

[e] A urinary tract infection caused by a *Candida* sp. that was treated with amphotericin bladder irrigations.

always require systemic antifungal therapy but only removal of the offending intravascular catheter (13). However, results of recent studies have indicated significant morbidity and mortality related to untreated candidemia (23, 29, 32). These studies have also failed to identify clearly which patients do not require antifungal therapy. Consequently, there has been a growing trend among clinicians to recommend a course of amphotericin B therapy for episodes of candidemia (5, 11, 23, 32), even in nonneutropenic hosts.

Although blood cultures may be negative in a substantial proportion of patients with invasive candidiasis, the early recognition and management of candidemia are important in order to reduce candidiasis-related morbidity and mortality. Current diagnostic limitations in defining the extent of candidal infections in patients with and without fungemia emphasize the importance of a careful clinical-pathologic correlation and long-term follow-up in the evaluation of new diagnostic techniques.

The Cand-Tec assay is based on a latex agglutination technique involving latex beads coated with serum from rabbits immunized with whole, heat-killed blastoconidia of *C. albicans*. The assay is apparently specific for an uncharTABLE 3. Cand-Tec titers in sera from patients with focal and disseminated candidiasis[a]

Patient no.	Age (yr)	Sex	Underlying disorder	Candidiasis site	Diagnosis	Titer	Serum creatinine (μmol/liter)
1	72	M	Aortic valve replacement, candidemia	Endocarditis	Valve culture	Neg	174
2	59	M	Bowel resection	Liver abscess	Autopsy	1:16	229
3	57	M	Multiple myeloma, candidemia	Myocardium, brain	Autopsy	1:2	70
4	63	F	Pelvic sepsis, prior candidemia	Vertebral osteomyelitis	Biopsy	Neg	72
5	67	M	CABG	Retinitis, pleuritis	Clinical, pleural fluid culture	1:8	77
6	59	F	Leukemia (AML), prior candidemia	Hepatic	Biopsy	1:4	46
7	50	M	Leukemia (AML), prior candidemia	Disseminated (liver, spleen, pancreas, kidneys)	Autopsy	Neg	118
8	20	F	BMT (ALL), candidemia	Disseminated (liver, spleen, CNS)	Autopsy	1:2	122
9	24	F	BMT (AML), candidemia	Disseminated (heart, lung, kidney, spleen, bowel)	Autopsy	1:8	547

[a] Abbreviations: M, male; F, female; CABG, coronary artery bypass graft; AML, acute myelogenous leukemia; BMT, bone marrow transplant; ALL, acute lymphocytic leukemia; CNS, central nervous system.

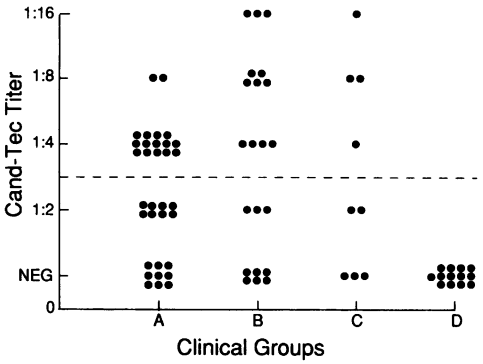

FIG. 1. Cand-Tec titers for clinical groups A (candidemia), B (negative controls), C (focal invasive or disseminated candidiasis), and D (healthy controls).

acterized, heat-labile fungal antigen which circulates in the sera of patients with invasive infections caused by *C. albicans*, *C. tropicalis*, or *C. parapsilosis* (17). The antigen is detectable without immune complex dissociation steps. This immunoassay was developed for the purpose of identifying patients with disseminated candidiasis rather than the subgroup of patients with only candidemia. Gentry et al. (17) applied the assay to 33 patients with disseminated candidiasis. The patient population was heterogeneous and included patients with leukemia, *Candida* endocarditis, and various other risk factors for opportunistic mycoses. In that study, disseminated candidiasis was defined as fever plus a positive blood or organ culture for *Candida* spp. By using that definition, 23 patients in that study could be considered to have been candidemic (defined as one or more positive blood cultures) but without clinical, microbiologic, or histologic evidence of invasion of other tissues. Positive titers of ≥1:4, in the absence of nonspecific agglutination, were obtained for 87% (20 patients) of the candidemic patients in that study compared with a sensitivity of 91% for the entire group. An unspecified number of cases of "line-related candidemia," "in the absence of apparent organ involvement," had negative titers. The latter patients appeared to have been excluded from the analysis. The specificities of the assay, when applied to healthy controls and various patient groups (including colonization by *Candida* sp., renal failure, and bacteremia), were 100 and 97%, respectively.

DeLozier et al. (10) reviewed the Cand-Tec assay results for 36 surgical patients with a diagnosis of "*Candida* sepsis." This patient population included 30 with one or more positive blood cultures and 4 with *Candida* cultured from at least three sites or from deep abscesses. For the 36 patients, the sensitivity and specificity of the assay were 94.4 and 100%, respectively, when a positive threshold titer of ≥1:4 was used. Although it was not specified, the sensitivity of the assay for the candidemia subgroup would have been at least 93.3% (i.e., if both false-negative test results had occurred in the candidemic patients). The specimens for the Cand-Tec assays were collected and the test result was obtained at an unstated interval of time before or after blood cultures had yielded *Candida* sp. in 15 (44%) and 19 (56%) of the patients, respectively.

Fung et al. (15) evaluated the Cand-Tec assay in sera from 6 patients with invasive candidiasis, as well as in sera from 18 other patients who were colonized with *Candida* sp. Only one patient was described as being candidemic; the peak titer in serum was positive at a titer of 1:8. In the same study, the LA-CADS (Immuno-Mycologics), which detects *Candida* mannan antigen, gave negative results for all 81 serum samples tested.

Burnie and William (8) reported their experience with the Cand-Tec assay for 30 patients with invasive candidiasis. By using a threshold titer of ≥1:4, positive tests were obtained for seven (78%) of the nine patients with evidence of invasive infection limited to candidemia. The specificity, as determined from a group of colonized patients, was 99%. Cabezudo et al. (9) reported a limited role for the Cand-Tec assay in the early diagnosis and prognosis of patients at high risk of invasive candidiasis. This series included 25 patients with candidemia (with two or more positive blood cultures, collected from separate sites at least 24 h apart) and 6 patients with transient candidemia (defined as a single positive blood culture and no other evidence of systemic infection). Similar results were obtained for both groups; by using a titer of 1:4, the sensitivity and specificity for candidemia were 64 and 72%, respectively. Several other studies either excluded patients with only candidemia (27) or presented the data without a separate analysis for patients with candidemia (20, 25, 31).

Despite those encouraging results, other investigators have had less favorable experience with the assay. Bailey et al. (2) found the Cand-Tec assay to have a sensitivity of 28% and a specificity of 100% when applied to a collection of serum specimens from 18 patients with disseminated candidiasis and 25 control serum specimens. Negative results were obtained for all three of their patients with candidemia. All 25 controls were healthy, and 5 patients were neutropenic.

Ness et al. (24) evaluated the Cand-Tec assay with sera from immunocompromised patients with hematologic malignancies or solid tumors. Of the 11 patients they studied, 4 fulfilled their criteria for invasive candidiasis on the basis of sustained candidemia, which was defined as two or more positive blood cultures obtained from two different sites at least 24 h apart. *Candida* antigen titers were positive in only one (25%) of these patients on days when blood cultures yielded *Candida* sp.

Escuro et al. (14) found the Cand-Tec assay to be unreliable for the diagnosis of deep candidiasis in neutropenic patients. Only three patients in their series could be considered to be candidemic without organ involvement; serum from one of the three patients (33%) was positive at a titer of ≥1:8. Lemieux et al. (22) evaluated the Cand-Tec assay in sera from patients with various forms of invasive candidiasis, including transient (≤48 h) or sustained candidemia. By using a titer of 1:8, the sensitivity and specificity for sustained candidemia were 44 and 100%, respectively. The results with the Cand-Tec assay for studies which permitted analysis of candidemic patients are summarized in Table 4.

For patients with documented invasive candidiasis, the sensitivity of the Cand-Tec assay has varied from 28 (2) to 94% (10) in sera from the various patient populations to which it has been applied. Similarly, for sera from candidemic patients the sensitivity and specificity have been variable. Some investigators have suggested that a positive titer for the Cand-Tec assay should be ≥1:4 (10, 17), whereas others have found the specificity to be unacceptably low at titers of 1:4 or less (15).

The results of earlier studies (8, 10, 15, 17) have suggested that the immunoassay may provide a rapid ancillary test for candidemia (as well as other forms of invasive candidiasis) that can be used in addition to lysis-centrifugation or standard blood culture techniques for sera from selected patients. However, the Cand-Tec assay proved to be unreliable

TABLE 4. Cand-Tec assay results in patients with candidemia

Reference	Yr	No. of patients[a]	Study patient population[b]	Titer[c]	Sensitivity (%)	Specificity (%)
17	1983	23	Mixed	1:4	87	97
8	1985	9	Mixed	1:4	78	99
2	1985	3	NS	1:4	0	100
15	1986	1	Mixed	1:8	100	98
10	1987	30	Surgical	1:4	>93	100
24	1989	4	Hem-onc	1:4	25	29
9	1989	6 (T)	Hem-onc, ICU	1:4	66	72
				1:8	17	80
		25 (S)	Hem-onc, ICU	1:4	64	72
				1:8	36	80
14	1989	3	Oncology	1:8	33	90
22	1990	6 (T)	Mixed	1:4	83	76
				1:8	67	100
		9 (S)	Mixed	1:4	67	76
				1:8	44	100
This study	1990	33	Mixed	1:4	49	43

[a] Abbreviations: T, transient candidemia; S, sustained candidemia
[b] Abbreviations: Mixed, mixed medical and surgical patients; NS, not specified; Hem-onc, hematologic malignancy and oncology; ICU, intensive care unit.
[c] Titer is the threshold titer used for a positive test result.

in sera from our candidemic patient population. By using a threshold positive titer of ≥1:4, the sensitivity and specificity were 49 and 43%, respectively. The positive and negative predictive values were 9 and 88%, respectively, for a selected high-risk population with an estimated candidemia prevalence of 10%. When the cutoff value for a positive test was set at ≥1:8, the sensitivity fell to 6% and the specificity increased to 62%. If any positive titer (≥1:2) was considered significant, then the sensitivity and specificity were 73 and 29%, respectively. Rheumatoid factor was excluded as the cause of false-positive results in all but 1 (from whom a serum specimen was not available) of 12 group B-negative controls with titers of ≥1:4.

The analysis was repeated by excluding patients with only one positive blood culture for *Candida* sp., since specimen contamination rather than true candidemia may have accounted for some of these cases. Similar results were obtained, with positive titers (≥1:4) occurring in 10 (48%) of the 21 patients (Table 1). In contrast to the results of a previous study (24), we found no relationship between the presence of renal impairment and a positive titer by the Cand-Tec assay.

Consistent with a report by Fung et al. (15), we found no positive results using the LA-CADS (Immuno-Mycologics) in any of the 106 tests that we performed.

An antigen detection immunoassay which is reliable for identifying patients with extensive candidal tissue invasion may not necessarily prove to be useful for diagnosis in patients with other forms of invasive candidiasis, such as limited focal infection (e.g., endophthalmitis) or candidemia (2, 3). The simple addition of *Candida* sp. to blood in vitro apparently does not result in detectable antigenemia by the Cand-Tec assay (11). It has been suggested that the Cand-Tec assay may detect a "host-processed" fungal antigen (11) or, possibly, a nonfungal host antigen which is cross-reactive with an antigen of *C. albicans* (3). The early stages of catheter-related candidemia may be analogous to the in vitro addition of *Candida* sp. to blood, possibly accounting for the absence of detectable antigenemia in many of our patients. Alternatively, candidemia may be associated with fungal antigenemia, and with an appropriate assay, its detection may have clinical utility.

The Cand-Tec assay appears to be unreliable for detection of disseminated candidiasis in patients with serious immunocompromise related to a hematologic malignancy (24). Our results were similarly disappointing for nine patients with focal invasive or disseminated candidiasis (Table 3) associated with a variety of underlying diseases.

Our findings indicate that neither of these two immunoassays plays a role in the identification of patients with candidemia. Further efforts will need to be directed toward the rapid diagnosis of candidemia in order to facilitate prompt initiation of systemic antifungal therapy.

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